REPRODUCTION
REVIEW

Negative effects of endocrine disruptor bisphenol A on ovarian granulosa cells and the protective role of folic acid

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Abstract

In brief: Bisphenol A (BPA) is a widely produced chemical, mostly used in the production of polycarbonate plastics, and can act as an endocrine disruptor. This paper focuses on the different effects of BPA on ovarian granulosa cells.

Abstract: Bisphenol A (BPA) is an endocrine disruptor (ED), widely used as a comonomer or an additive in the plastics industry. It can be found in food and beverage plastic packaging, epoxy resins, thermal paper and other common products. To date, there have only been several experimental studies to have examined how BPA exposure affects human and mammalian follicular granulosa cells (GCs) *in vitro* and *in vivo*; the collected evidence data show that BPA negatively affects the GCs by altering steroidogenesis and gene expression, inducing autophagy, apoptosis and cellular oxidative stress through reactive oxygen species production. Exposure to BPA can also lead to abnormally constrained or elevated cellular proliferation and can even reduce cell viability. Therefore, research on EDs such as BPA is important as it provides some important insights into the causes and development of infertility, ovarian cancer and other conditions related to impaired ovarian and GC function. Folic acid, a biologic form of vitamin B9, is a methyl donor that can neutralize the toxic effects of the BPA exposure and is, as a common food supplement, an interesting option for researching its protective role against ubiquitous harmful EDs such as BPA.

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Introduction

Infertility, defined as the inability to conceive naturally, is an ongoing problem among many young couples worldwide (10–15% globally), and the causes of infertility often remain unknown (Pivonello *et al.* 2020). Problems with fertility can affect either partner, that is, the male, the female or both. The most common causes of female infertility are endometriosis, problems with the fallopian tubes, ovarian infertility such as polycystic ovary syndrome (PCOS), low ovarian reserve, poor ovarian response to hormonal stimulation and premature ovarian insufficiency (POI), also called early menopause (Tamrakar & Bastakoti 2019). In some cases, where the cause of infertility has not been identified, it is known as idiopathic infertility. The causes and risks of infertility are often endogenous, but there are also several exogenous factors that can affect fertility. These include sexually transmitted infections, such as chlamydia, smoking, alcohol and other environmental factors, such as continuous exposure to harmful chemicals or metals (Gallo 2022, Sharpe & Franks 2002). It is also possible that endocrine disruptors play a role.

Endocrine-disrupting chemicals or endocrine disruptors (EDs) are a special class of chemicals that can disrupt and affect the endocrine system and can thus be harmful to humans and animals (Kabir *et al.* 2015). With all the evidence collected, EDs show the ability to disrupt the endocrine system on many levels and dysregulate the expression of hormones and their respective receptors, which can lead to different pathological outcomes, including female infertility (Jozkowiak *et al.* 2022, Land *et al.* 2022 , Bellavia *et al.* 2023).

Bisphenol A (BPA) is one of the most common and ubiquitous EDs and is widely used in the making of many consumer products, including food packaging and bottles (Konieczna *et al.* 2015, Chen *et al.* 2016). The most important sources of BPA are food and beverages, as BPA leaches from food and beverage packaging, especially after heating (Acconcia *et al.* 2015, Konieczna *et al.* 2015). BPA in humans is metabolized in the liver and later excreted by urine (Pivonello *et al.* 2020). Several studies have concluded that BPA negatively affects the reproductive system largely through binding to estrogen receptors (ERs) and thus stimulating estrogen function or via oxidative stress-related damage (Tsutsumi 2005). BPA can affect ovarian function, especially steroidogenesis and follicular growth, and even ovarian morphology (Meli *et al.* 2020).

BPA can affect various phenotypes that are regulated by the natural hormone, estrogen, which is produced in the ovaries and is responsible for the development

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and regulation of the female reproductive system and secondary sex characteristics. BPA shows a high affinity for ERs because it expresses the phenol group similar to estradiol (E2) and exhibits estrogen-mimicking behavior (Ma *et al.* 2019, Stavridis *et al.* 2022, Tarafdar *et al.* 2022). BPA has been found to interact with ERs (Wehbe *et al.* 2020) located both in the cell membrane (G-protein-coupled receptor GPR30) and in the cytoplasm/nucleus (ERα (ESR1) and ERβ (ESR2)), which are encoded by genes *ESR1* and *ESR2* and distributed in different cells and tissues: ERα in hypothalamus, endometrium and breast cancer cells and ERβ in ovarian granulosa cells (GCs), brain, kidney, bone, heart, lungs, intestinal mucosa and endothelial cells. The BPA activity includes changes of hypothalamus ERα and ERβ expression at relatively low doses (260 µg/kg body weight (BW)/day and lower), thus indicating that even concentrations, lower than the estimated daily intake doses, can affect the organism. Additionally, more studies have evidenced the ability of BPA to bind G-protein-coupled estrogen receptors (GPR30) and interfere with estrogen-activated signaling pathways (Nadal *et al.* 2018, Stavridis *et al.* 2022, Chevalier & Fénichel 2015). BPA binds to estrogen and GPCRs with affinity, which is approximately 10,000 times lower than that of the steroid hormone E2; however, its actions are not always dose dependent and as such hard to predict (Wetherill *et al.* 2007).

All this suggests that BPA could potentially arouse estrogen function and contribute to the pathogenesis of various diseases of the female reproductive system by disrupting the hypothalamic–pituitary–gonadal (HPG) axis (Rebuli *et al.* 2014). This way, BPA exposure may lead to alterations in female reproductive hormone levels (Miao *et al.* 2015) and cause steroid hormone imbalance, PCOS, menstrual cycle disorder, POI, and lowered ovarian reserve (Souter *et al.* 2013, Li *et al.* 2021, Shi *et al.* 2021).

The ED BPA has the capacity to bind androgen receptors (ARs) and inhibit androgen function, and, knowing the principle of HPG axis (Fig. 1), it may interfere with gonadotropin-releasing hormone (GnRH) released from hypothalamus (Xu *et al.* 2018), gonadotropins such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) released from pituitary, and progesterone synthesized by the adrenal cortex as well as the ovaries (also ovarian corpus luteum during pregnancy) which are all important for different aspects of reproductive health and function in women (Miao *et al.* 2015, Vahedi *et al.* 2016 , Chen *et al.* 2018, Lazúrová *et al.* 2021, Li *et al.* 2021). The pulsatile release of GnRH from the hypothalamus induces the release of LH and FSH from the pituitary which act on ovarian follicles to release progesterone and estrogen; E2 is one of three estrogens produced that regulates the release of LH and GnRH by a negative feedback action (Adachi *et al.* 2007). Because BPA possesses the ability to mimic the action of E2, it can bind to

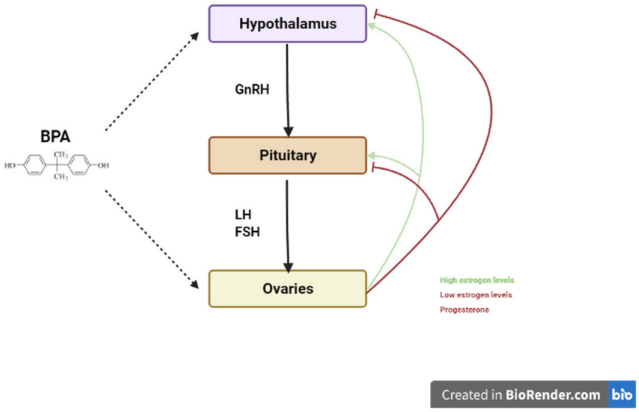

Figure 1 The effect of BPA on the hypothalamus–pituitary–gonadal axis. BPA may bind to hypothalamic and ovarian granulosa cell estrogen receptors (ERα and ERβ, respectively) and disrupt the release of GnRH from hypothalamus and gonadotropins (FSH and LH) from the pituitary. Consequently, it affects the steroid hormone feedback at all levels which can cause alterations within the female reproductive cycle. BPA, bisphenol A; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

ERα receptor (Fang *et al.* 2000) and in this way disrupt the functioning of the hypothalamus (kisspeptin KISS1) and pituitary gland; moreover, BPA can also target LH and FSH receptors (LHCGR and FSHR), several growth factors and proteins/enzymes involved in the process of steroidogenesis in the female reproductive tract (Mukhopadhyay *et al.* 2022).

In women with PCOS, urinary BPA levels negatively associate with ovarian steroid hormones such as E2, testosterone, free androgen index, and testosterone, thus suggesting a possible suppressive effect of BPA on ovarian steroidogenesis (Lazúrová *et al.* 2021). In another study, increased urinary BPA levels in PCOS patients tended to be related to serum anti-Müllerian hormone (AMH) and day-3 FSH levels, while there was no association with inhibin (Zhou *et al.* 2016). It was evidenced that BPA shows the ability to dysregulate AMH and AMHRII expression in different cells and tissues, including oocytes and cumulus–oocyte complex, which indicates its damaging influence on female reproduction within highly hormone-sensitive follicular environment and ovarian reserve (Zhou *et al.* 2016, Czubacka *et al.* 2021, Saleh *et al.* 2021).

In addition, BPA can affect and disrupt the thyroid hormone system, where due to similar structure, BPA can bind to thyroid hormone receptor (TR; particularly TRα and TRβ) positioned in proximity to the cell nucleus, acting as a transcription repressor, AR, pregnane X receptor and peroxisome proliferator-activated receptor (Marino *et al.* 2012, Acconcia *et al.* 2015). So, BPA acts antagonistically by inhibiting TR-induced transcription of thyroxine and triiodothyronine-responsive genes (Kim & Park 2019). In addition, BPA can also affect transcription of factors, involved in thyroid hormone synthesis (TPO,

NIS, Tg and PAX8) and thyroid development (*Slc5a5*) (Gorini *et al.* 2020).

EDs can be found in almost every industry field, agriculture and retail. Exposure can happen through ingestion, inhalation of air particles and/or gases and dermal absorption (Gore *et al.* 2014, Rashid *et al.* 2020). EDs can accumulate in fat tissues and remain there for a long time. The effect that EDs have on human health can often remain hidden and is not evident until later in life (Rattan *et al.* 2017). There are many different animal studies, epidemiological studies and clinical reports that demonstrate how EDs affect male and female reproduction, thyroid functioning, nervous system, metabolism and related comorbidities such as obesity and diabetes (Crain *et al.* 2008, Kabir *et al.* 2015, Bonde *et al.* 2016, Kiess *et al.* 2021, Sang *et al.* 2021). It is worrying that research on animal models (e.g. rats and mice) has shown that perinatal exposure to low doses of BPA during the critical period of hypothalamic sexual differentiation modifies the activity of the HPG axis in the offspring, with advanced puberty and consequences for later life in adult animals (Sadowski *et al.* 2014, Moustafa & Ahmed 2016, Ma *et al.* 2017, Oliveira *et al.* 2017 , Ahsan *et al.* 2018, Xu *et al.* 2018). Also in humans, some findings suggest that BPA exposure (possibly during pregnancy) appears to be related to an earlier age at onset of puberty (precocious puberty) in girls, especially in those who are obese (Supornsilchai *et al.* 2016).

The harmful effects and toxicity of BPA are connected with both oxidative stress and stress markers (Gassman 2017) and through activating nuclear, orphan (aryl hydrocarbon receptor) and noncanonical steroid hormone receptors, most notably two estrogen-type receptors, ERα and ERβ, which, upon binding, change conformation and travel into the nucleus or activate extranuclear, nongenomic pathways; they bind to DNA to regulate the activity of different genes (as a DNA-binding transcription factors). However, they also have additional functions independent of DNA binding. Different studies have shown that the increased production of reactive oxygen species (ROS) majorly contributes to BPA toxicity by disrupting cellular redox balance, depleting antioxidant reserves and thus causing mitochondrial distress and changes in cellular signaling pathways, which can lead to inflammation or apoptosis (Wetherill *et al.* 2007, Moon *et al.* 2012). According to the European Food Safety Authority (EFSA) report (2015), the current daily dose of BPA exposure (tolerable daily intake (TDI): 4 μg/kg BW/day and specific migration limit: 0.05 mg/kg food) does not present a threat to human health (Almeida *et al.* 2018).

This review aims to offer some new connections and insights regarding the consequences of damage caused by BPA to ovarian tissue and function, with special emphasis on follicular GCs which are significantly involved in steroidogenesis. This review will focus on studies describing how BPA affects human healthy and malignant (immortalized) and animal GCs at their molecular level since they are closely associated with the development and function of the ovaries, follicles and oocytes and consequently with female (in)fertility.

Methods

This review is based on studies that were retrieved by systematic search in PubMed (MEDLINE) using the following keyword combinations: ''(BPA OR Bisphenol A AND Granulosa)'' with 67 results, ''(BPA OR Bisphenol A AND Fertility)'' with 354 results, ''(BPA OR Bisphenol A AND Cumulus)' with 19 results, ''(BPA OR Bisphenol A AND folic*)' with 27 results and ''(BPA OR Bisphenol A AND Oxidative stress)'' with 634 results. Only experimental studies and existing review articles published in English from 2000 to 2022 were selected. Other criteria included experimental studies in humans and mammals and studies that only analyzed the stand-alone effects of BPA. We tried to determine the possible negative effects of BPA on ovarian function, especially follicular GCs.

Findings

We found a number of negative effects of BPA on ovarian function, including GCs, in both humans and animal models. To better understand the negative effects of BPA on the ovaries, we provide some basic knowledge on the ovary, from folliculogenesis/oogenesis, follicles, oocytes and follicular GCs to steroidogenesis.

Folliculogenesis, follicles, oocytes, GCs and steroidogenesis

The most important ovarian unit is the ovarian follicle, a structure of tightly packed somatic cells, mostly granulosa and theca cells, with an oocyte inside (Baerwald *et al.* 2012). Each follicle has the potential to mature and someday release a mature oocyte. Ovarian follicles exist at several developmental stages, starting with small primordial follicles, which consist of an immature oocyte, surrounded by a single layer of flattened GCs (Yamada & Satoh 1997). Primordial follicles are formed during fetal development, and, as the reserve of immature oocytes, they are the most abundant follicular stage in the ovary. Located in the cortical periphery, most of them never develop beyond the primordial phase (Wallace & Kelsey 2010, Monniaux *et al.* 2019). The exact mechanism that dictates the transition to the next follicular stage is still unknown (Monniaux *et al.* 2019). The primordial follicle stage is followed by transformation into primary follicles that are 10 times larger. An increase in size is a consequence of a growing oocyte inside the follicle and a change in the shape of the surrounding GCs that are becoming metabolically and secretorily active in this stage. They communicate with each other through special tight and gap junctions that allow them to

exchange small molecules (Baerwald *et al.* 2012). In this phase, a thick glycoprotein-rich layer, called the zona pellucida, develops around the oocyte and borders the GC layer. During the growth of the follicle, GCs start to multiply, and the previously single-cell granulosa layer becomes thicker, which depicts a transformation into the secondary follicle. In this stage, theca cells from the surrounding environment merge with the outer layer of a secondary follicle and create a new peripheral layer called the thecal layer (Fortune *et al.* 2000). The thecal layer is, in contrast to the avascular granulosa layer, intertwined with blood vessels, enabling the delivery of nutrients into the follicle. Some secondary follicles develop further into tertiary follicles with a newly formed fluid-filled cavity called the antrum. Antral fluid contains many different protein and steroid hormones, enzymes and ions (Fortune *et al.* 2000). The granulosa layer of tertiary follicles consists of several layers of GCs, and the thecal layer has now developed into two different layers, the theca interna and theca externa. The oocyte is now protruding into the antral cavity, surrounded by a special layer of GCs called the cumulus oophorus (Monniaux *et al.* 2019). GCs are very important as they feed the oocyte and direct it in its growth and development inside the follicle. Tertiary follicles can be divided into three subgroups, depending on their stage of maturity: early tertiary follicles, late tertiary follicles and the most mature late or Graafian follicles. During each menstrual cycle, only one Graafian follicle achieves final maturation and evolves into the preovulatory follicle, while the other follicles die (atresia) (Monniaux *et al.* 2019).

Steroidogenesis in follicular cells

Ovarian follicles produce and secrete three groups of steroid hormones that are essential for successful reproduction (Bremer & Miller 2014). These three groups of hormones are estrogens, androgens and progestins, and all of them have the same precursor, cholesterol. Cholesterol enters theca cells through receptor-mediated endocytosis or is *de novo* synthesized in theca cells (Miller & Auchus 2011). Steroidogenic acute regulatory (StAR) protein then manages cholesterol transport to the mitochondrial matrix, where the production of steroid hormones occurs (Soccio & Breslow 2003). The production and synthesis of steroid hormones is called steroidogenesis and consists of multiple steps of specific enzymatic modifications of steroid molecules. Each type of ovarian follicular cell has its own role in steroidogenesis, and each type of cell synthesizes specific steroidogenic enzymes. Glandular cells of the theca interna use cholesterol with several intermediate precursor enzymes, such as cytochrome P450 family 17 subfamily A member 1 (CYP17A1), 3β-hydroxysteroid dehydrogenase (HSD3b), 17a-hydroxylase-17,20-desmolase (CYP45017a) and 17β-hydroxysteroid dehydrogenase (HSD17b), to produce androstenedione, which then passes into the GC layer (Marti *et al.* 2017). GCs convert androstenedione to testosterone, and the latter is then converted to E2 by aromatase (CYP450arom; CYP19A1). E2 later diffuses into the thecal layer and enters the bloodstream from there. This is called the two-cell model of ovarian steroid hormone production (Jones & Lopez 2006). GCs of preantral follicles also produce AMH, which regulates follicular development by preventing the maturation of early follicles, recruiting and selecting dominant follicles and inhibiting others by preventing their access to FSH (Bedenk *et al.* 2020). As a biomarker, AMH is crucial for assessing the relative size of ovarian reserve and even for measuring some aspects of ovarian function, such as PCOS and POI (Bedenk *et al.* 2020).

Sources of human GCs and immortalized GC lines for research

An interesting source of GCs is the follicular fluid, obtained by ultrasound-guided aspiration of ovarian follicles to obtain oocytes for *in vitro* fertilization (IVF) after hormonal stimulation of the ovaries (Beschta *et al.* 2021). In assisted conception in clinical practice, follicular fluid is usually discarded after oocyte removal but can be a valuable source of GCs for research. GCs can be isolated from follicular fluid on the basis of the expression of various markers, such as FSHR, cytochrome P450 family subfamily A member 1 (CYP19A1), AMH, nuclear receptor subfamily 5 group A member 2 (NR5A2) and mitochondria localized glutamic acid-rich protein (MGARP) (Kossowska-Tomaszczuk *et al.* 2009, Hatzirodos *et al.* 2015).

More studies have been performed on the GC line COV434, an immortalized human GC line with the majority of the essential functions preserved; the addition of FSH stimulates cellular growth, proliferation and synthesis and secretion of estrogens (Zhang *et al.* 2000). It was established from a primary human granulosa tumor from a 27-year-old woman with metastatic GC carcinoma. GCs possess three specific cellular functions: the production of estrogen in response to FSH, the expression of specific biomarkers of apoptosis necessary for inducing follicular atresia and the ability to communicate with other follicular cells. Possessing them all, the COV434 cell line is crucial for ovarian and follicular research *in vitro* (Zhang *et al.* 2000).

The KGN cell line is another human ovarian granulosa-like tumor cell line established from a patient with invasive ovarian GC carcinoma. The KGN cell line maintains most of the distinct GC functions, most notably steroidogenesis, normal apoptosis and FSH receptor expression. This cell line thus allows the study of different aspects of human GCs *in vitro* (Nishi *et al.* 2001).

Animal models, especially mammalian models, are essential for biological research because of extraordinary

physiological and anatomical similarity to human (Barré-Sinoussi & Montagutelli 2015). Most mammals and humans show very similar folliculogenesis, oogenesis and embryonic development. Animal GCs (and other cells of the reproductive system) are very important for research because they provide reliable and normally functioning primary cellular models, which offer, in comparison to hormone-treated luteinized human GCs and immortalized GCs, the closest approximation to *in vivo* conditions (Sabry *et al.* 2022). Most researches used bovine, ovine, porcine and murine GCs.

Bisphenol A

One of the most extensively studied EDs is BPA. BPA, 4,40-dihydroxy-2,2-diphenylpropane, is a commonly used organic compound and was first synthesized as a synthetic estrogen (Acconcia *et al.* 2015). BPA has two phenol groups and is mostly used as a comonomer in the production of polycarbonate plastic and epoxy resins as a flame retardant and antioxidant. It can be found in many different consumer products, mostly food packaging, plastic bottles, nursing bottles, lenses, dental materials, thermal paper and so on. BPA-containing polycarbonate plastic materials are strong, tough, transparent and highly resistant to heat- and cold-induced degradation and acidic environments (Vasiljevic & Harner 2021).

Because of its hormone-mimicking features, BPA can be placed in a group of chemicals called xenoestrogens, more specifically EDs, as they imitate the body's own 17β-E2 actions by binding to ERs. Although its effect is not proven to be very strong, its wide prevalence raises many concerns. Most people are frequently, even daily, exposed to low concentrations of BPA. Most ingested BPA is through food and drink, as BPA is used in epoxy coatings and plastics. Other sources of human exposure are clothing, thermal paper (e.g. purchase invoices), dental fillings, other devices, air and dust particles from which BPA is absorbed through skin contact (Geens *et al.* 2012). BPA can be detected in human serum, plasma, saliva, hair, amniotic fluid and cord blood; after being converted to more water-soluble forms through glucuronidation, BPA leaves the body through the urine, with a half-life of approximately 6 h (Huang *et al.* 2018). BPA can also accumulate in different human and animal tissues, affecting and altering their functions (Cimmino *et al.* 2020).

An assortment of several independent studies have reported an estimated dietary daily BPA intake of approximately 0.4–4.2 μg/kg BW/day (Ribeiro *et al.* 2017); this assessment puts human daily exposure in the range of 'low-dose' exposure with no documented side effects (the lowest no observed adverse effect level concentration is 20 mg/kg BW/day in rats) (Ribeiro *et al.* 2017). The EFSA estimates the maximum TDI of BPA to be approximately 50 μg/kg BW/day with possible reevaluations in the future (Ćwiek-Ludwicka 2015). Daily exposure variates between different countries, with Italy being the country with maximal estimated BPA intake and Tunisia the country with the lowest BPA daily intake (Huang *et al.* 2017). BPA intake also depends on dietary customs such as higher consumption of canned foods, which were related to higher urinary levels of BPA (Liu *et al.* 2018, Hartle *et al.* 2022) and occupational groups including thermal paper factory, plastic factory and epoxy resin manufacturing factory workers with higher urinal BPA concentrations than in control groups (Hines *et al.* 2017, Kouidhi *et al.* 2017, Ribeiro *et al.* 2017, Bousoumah *et al.* 2021).

However, trends of exposure are changing, mostly due to the general transition to BPA analogues in the production of baby bottles and food packaging, but the temporal trends of exposure vary globally (e.g. comparison of temporal trends of BPA exposure between Los Angeles and Beijing showed steady decline of exposure in Los Angeles through the years, while no significant temporal trend was observed in Beijing (Lin *et al.* 2020)).

Other BPA analogs, such as bisphenol F (BPF) and bisphenol S (BPS), are also known and increasingly used as 'safer alternatives' but are much less researched. While BPS is usually used in epoxy glues and thermal receipt papers, BPF has a broader spectrum of use; it can be used in the dental industry, prosthetics, food packaging, plastic industry and so on. (Chen *et al.* 2016). Human exposure to bisphenol analogs is similar to BPA exposure, that is, oral and dermal, mostly through food and skin contact. Although data and studies on BPA analogs are still limited, reported results show a wide range of different toxic effects, such as cytotoxicity, genotoxicity, endocrine disruption and impaired reproductive health, suggesting that bisphenol analogs might not be as safe as previously thought (Rochester & Bolden 2015). However, more research is still needed to prove the estimated harmful effects of bisphenol analogs and classify them as xenoestrogens.

General health effects of BPA

In 2017, the European Chemical Agency decided that BPA should be classified as a chemical of high concern due to its role as an ED. Many studies on animal models have found strong connections between EDs and different metabolic diseases or altered body functions, as they imitate human estrogen 17β-E2 by binding to ERs and triggering a cellular response (Alonso-Magdalena *et al.* 2012). BPA works through genomic and nongenomic pathways, which can be triggered by nonspecific dose responses (Alonso-Magdalena *et al.* 2012). Nonmonotonic dose responses (NMDRs) are doses where the slope of the dose–response curve pattern is not just increasing or decreasing with dose but changes direction within the range of examined doses (Vandenberg *et al.* 2012). There are few examples how

different doses of BPA trigger different cell responses: (1) through binding to ERβ, low doses of BPA alter glucose-induced insulin secretion and decrease the expression of genes encoding calcium channels, decreasing calcium influx in mouse pancreatic β cells, yet activating said genes at high doses through ERβ and ERα; (2) at very low doses, BPA binds to GPR30/G protein-coupled estrogen receptor 1 (GPER), thereby inducing fast-acting intracellular signaling but with doses increasing, BPA first binds to nuclear ERs and later, at really high concentrations, to ARs and TRs, altering their transcriptional activity (vom Saal & Vandenberg 2021). By binding to different receptors, BPA can affect many normal body functions, including causing obesity via thyroid dysfunction (Rezg *et al.* 2014, Heindel *et al.* 2017), altering thyroid function by binding to TRs (Andrianou *et al.* 2016) and many more actions, even affecting male and female fertility (Maffini *et al.* 2006, Radwan *et al.* 2018).

BPA can also affect some other diseases, such as breast and ovarian cancer (Guo *et al.* 2020, Segovia-Mendoza *et al.* 2020, Sang *et al.* 2021, Wan *et al.* 2021), colon cancer (Chen *et al.* 2015, Jun *et al.* 2021), respiratory diseases (Gascon *et al.* 2015, Wu *et al.* 2021), cardiovascular diseases (Moreno-Gómez-Toledano *et al.* 2021, Naomi *et al.* 2022), insulin resistance and type 2 diabetes mellitus (Soundararajan *et al.* 2019, Wade *et al.* 2020, Farrugia *et al.* 2021), obesity (Wu *et al.* 2020) and depression (Perera *et al.* 2016, Wiersielis *et al.* 2020).

Negative impacts of BPA on ovarian function and infertility

More research groups have analyzed the possible effects of BPA on female fertility and the correlation between serum and urine BPA levels and infertility (Ziv-Gal & Flaws 2016). The results from these studies show that women who struggle with infertility have higher serum BPA levels than those of fertile women (Ziv-Gal & Flaws 2016, Pivonello *et al.* 2020). Furthermore, BPA levels are also associated with poorer oocyte maturation rates, fertilization outcomes and embryo quality and can thus interfere with IVF treatments in infertile patients (Mok-Lin *et al.* 2010, Bloom *et al.* 2011). The exact reason for this is still unknown, and not all studies even agree on this association (Mínguez-Alarcón *et al.* 2015). The resulting mismatch might be due to differences in the sample size and health status among participating women. A study on mice has shown that BPA interrupts the normal cell cycle and upregulates atresia genes in mouse antral follicles, which may cause inhibited follicular growth and even follicular atresia (Peretz *et al.* 2012), which could be a major factor in decreased fertility. Some studies have also suggested a possible connection between BPA toxicity and PCOS; BPA is believed to worsen PCOS pathogenesis through various mechanisms within the HPO axis (Rutkowska & Rachoń 2014, Kechagias *et al.* 2020).

BPA toxicity on human follicular GCs

One of the most likely targets of BPA action in the ovaries are GCs in the follicles. This has been demonstrated in both immortalized GC lines and GCs derived from follicular fluid from the IVF process (Fig. 2). Let us summarize some of these findings.

Immortalized GC lines COV434 and KGN

Many studies have shown that BPA adversely affects immortalized GCs in a great variety of ways. We will describe some of them in the following section.

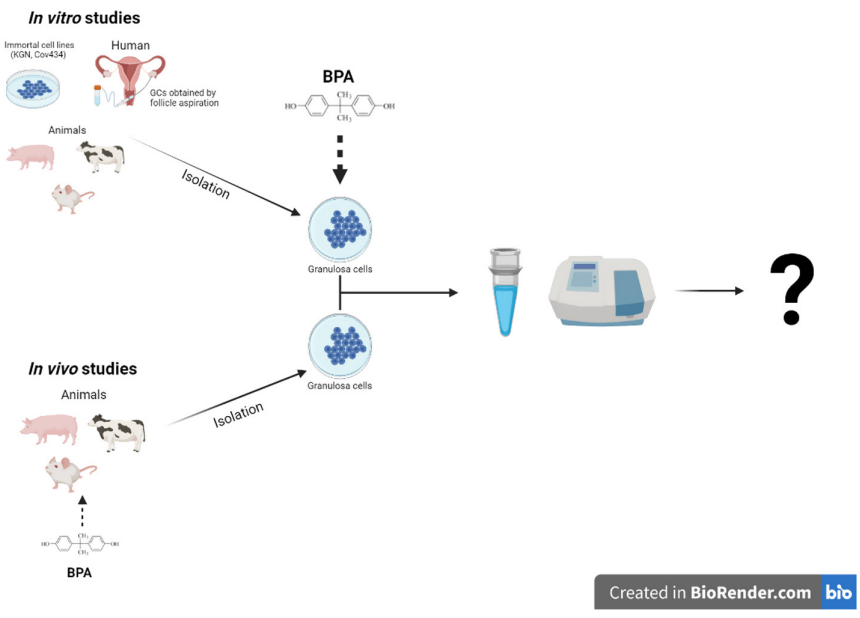

Figure 2 The flowchart of *in vivo* and *in vitro* experiments of BPA effects on ovarian granulosa cells. *In vitro* studies mostly use animal GCs, human immortalized GC cell lines and human GCs, obtained by the follicle aspiration in the IVF procedure. GCs are isolated from the ovaries and then exposed to BPA *in vitro* and later analyzed on toxic effects. *In vivo* studies are performed on different animal models, such as rats, mice, pigs and cows, treated with BPA. GCs from treated animals are later isolated and analyzed on their properties.

Cell viability and proliferation

BPA affects GC viability in a concentration- and time-dependent manner. Studies have used different methods and BPA concentrations to assess the effect of BPA exposure on immortalized cell line viability and have obtained similar results. Exposure to higher concentrations of BPA can significantly decrease cell viability in a time-dependent manner, especially concentrations of 100 µmol/L and higher (Qi *et al.* 2020, Bujnakova Mlynarcikova & Scsukova 2021, Huang *et al.* 2021, Liu *et al.* 2021*a*). Some previous studies from the same authors used lower concentrations (up to 10 µmol/L) to evaluate how BPA exposure influences cell viability and obtained no significant results (Mlynarcikova & Scsukova 2020). One study concentrated on assessing environmental concentrations of BPA (0.5, 5, 50 and 500 µg/L) and shorter times of exposure (6 h) and concluded that environmental levels of BPA do not decrease GC viability (Shi *et al.* 2021*a*). However, human exposure to BPA is constant, and future studies should focus on how environmental doses of BPA affect GCs over longer periods of time. It is also important to add that GC lines represent malignant cells from granulosa tumors and may act differently in response to BPA exposure.

It is not exactly certain how and if BPA treatment affects GC proliferation, as studies present different results. Bujnakova Mlynarcikova & Scsukova (2021) and Kwintkiewicz and colleagues both examined how standard concentrations (up to 100 µmol/L) affect cell proliferation and obtained opposing results. One study showed no significant effect (Bujnakova Mlynarcikova & Scsukova 2021), while another found that BPA inhibits GC proliferation in a dose-dependent fashion, with a maximum inhibition of 92 ± 2% at 100 µM BPA (Kwintkiewicz *et al.* 2010). Interestingly, lower, environmental doses of BPA (0.5 and 5 µg/L; 10 and 50 nM) sometimes even promote GC proliferation (Hoffmann *et al.* 2017, Shi *et al.* 2021). BPA exposure can affect cell morphology and function, as it significantly increases lipid droplet concentrations and their volumes (Rajkumar *et al.* 2021) or promotes nuclear and endoplasmic reticulum enlargement due to increased proliferation (Shi *et al.* 2021*a*). The increase in the lipid droplet number might be due to changes in cholesterol transport or to dysfunction in the fusion of autophagosomes and lysosomes (Rajkumar *et al.* 2021).

Steroidogenesis

Along with inducing oxidative stress and cellular apoptosis, BPA exposure may alter steroid hormone synthesis and metabolism *in vivo* and *in vitro* and cause steroid hormone imbalance, PCOS and disorders of the estrus cycle in animals. Based on the findings in GC lines thus far, the results can vary. KGN cells exposed to different BPA concentrations (0.5, 5, 50 and 500 µg/L) showed a significant decrease in the production of progesterone and E2 and the E2/testosterone ratio in a dose-independent manner; lower concentrations attenuated E2 levels, while higher concentrations had no effect (Qi *et al.* 2020). Lin and colleagues reported a dose-dependent effect of BPA exposure on impaired E2 production (Lin *et al.* 2021*a*), while another study described how only the lowest and highest BPA doses significantly decreased the levels of progesterone and the E2/testosterone ratio, whereas the middle two concentrations had no significant effect (Shi *et al.* 2021*a*). NMDRs go against typical dose–response relationships and are frequently associated with EDs. NMDRs can be explained by opposing effects, influenced by multiple receptors with different affinity or negative feedback loops with an increasing dose (Lagarde *et al.* 2015). Different experimental results can be a method- and dose-dependent consequence, with low conclusive value; more studies on GC cell lines should be executed in the future, with emphasis on method and protocol standardization.

It is not well understood how BPA influences steroid synthesis; some of the mechanisms are connected with BPA upregulating or downregulating the expression of steroidogenesis-related genes. Studies show opposing results regarding the influence of BPA on specific gene expression; Liu and colleagues (2021) discovered that in KGN cells, low concentrations of BPA (10^{-11} to 10^{-8} M) increase the expression of forkhead box L2 (*FOXL2*), a transcription factor involved in ovarian function and development. BPA also elevates aromatase (*CYP19A1*) expression and consequently E2 production (Kwintkiewicz *et al.* 2010) while inducing β-catenin (*CTNNB1*) expression in the so-called BPA-CTNNB1-FOXL2-CYP19A1-E2 signaling cascade (Liu *et al.* 2021*b*). Different outcomes of BPA exposure are presented in an older study from 2012 in which higher concentrations of BPA (50 µM) downregulated *CYP19* gene transcription and lowered CYP19 activities (Watanabe *et al.* 2012) or had no significant effects on *CYP19* at all (Shi *et al.* 2021*a*). The differences in the results might be a consequence of different exposure concentrations and methods or materials used to analyze the BPA influence. BPA exposure can decrease ferredoxin and ferredoxin reductase expression in a dose-dependent fashion. Both of these genes regulate progesterone synthesis in GCs. Exposure to BPA can also inhibit the expression of the testosterone-related genes hydroxysteroid 17-beta dehydrogenase 2 and 3 (*HSD17B2* and *HSD17B3*) and the E2 production-related gene hydroxysteroid 17-beta dehydrogenase 1 (*HSD17B1)* while simultaneously upregulating p450 oxidoreductase expression (Shi *et al.* 2021*a*). It has been shown that BPA does not affect *GPR30* mRNA expression but stimulates KGN cell proliferation and activity by binding to it (Hoffmann *et al.* 2017). BPA does not appear to affect the expression

of nuclear receptors, which might be important targets of BPA (Mlynarcikova & Scsukova 2020).

Gene expression

Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are important regulators of blood vessel development and angiogenesis produced by GCs. These factors are important for normal ovarian functioning and physiology, and many pathological conditions are related to VEGF and PDGF dysregulation. BPA significantly decreases the *VEGF* and *PDGF* expression in the COV434 cell line, although the mechanism of action has not yet been researched (Bujnakova Mlynarcikova & Scsukova 2021). Exposure to BPA also decreases the expression of the autocrine and paracrine protein chemerin and its receptor G protein-coupled chemokine-like receptor 1 (CLKMR1) in these cells (Hoffmann *et al.* 2018) and causes overexpression of peroxisome proliferator-activated receptor gamma (*PPARG*) (Kwintkiewicz *et al.* 2010).

Oxidative stress

As an ED, BPA impairs cellular redox homeostasis by inducing oxidative stress by decreasing antioxidant enzyme activity and altering the oxidation balance in mitochondria and cells. BPA exposure at higher concentrations significantly increased cellular ROS levels, causing a decrease in the antioxidant activity of the selected antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione synthase (GSH) and mitochondrial SODs ($P < 0.05$) in the KGN and COV434 cell lines (Huang *et al.* 2020, Mlynarcikova & Scsukova 2020). Exposure to BPA thus increases the levels of ROS in KGN cells by reducing intracellular antioxidant capacity and increasing cellular Ca^{2+} concentrations, which induce apoptosis. Huang *et al.* went even further and expounded the mechanism of BPA-related apoptosis. They studied whether and how inhibitors of associated ERs restrict apoptosis induced by BPA exposure. They discovered that treatment with G15, a GPER inhibitor, significantly prevented KGN cell apoptosis and influenced ROS and Ca^{2+} levels, while the ERα and ERβ inhibitors methylpiperidinopyrazole (MPP) and PHTPP had no effect. These studies show that BPA-induced oxidative stress causes KGN cell apoptosis through a GPER-dependent pathway and through increasing Ca^{2+} accumulation. BPA in high concentrations also increases markers of nucleic acid damage, such as 8-OHdG, malondialdehyde (MDA), an indicator of lipid damage, and protein carbonyls, which indicate protein damage (Huang *et al.* 2020). Macromolecular damage caused by BPA-induced oxidative stress is one of the triggers of cellular apoptosis, and GC apoptosis is one of the main factors of follicular dysfunction and atresia. Further studies with lower exposure concentrations are needed to better assess how even environmental BPA concentrations affect GCs and follicles.

Autophagy

Lin *et al.* studied how BPA affects KGN cells, an ovarian GC line, with respect to autophagy, apoptosis and endocrine disruption. KGN cells were treated with different concentrations of BPA (1, 10 or 100 nM) for different times (24, 48 or 72 h) and later analyzed with various methods to assess apoptosis and autophagy. BPA-induced cell apoptosis was measured by flow cytometry and TUNEL assays. GC autophagy was examined by immunofluorescence staining and western blotting after 48 h of culture. The 10 and 100 nM BPA exposure groups showed significant increases in the autophagic markers microtubule-associated protein 1A/1B light chain 3B and Beclin 1. Western blot analysis also showed significantly increased levels of 5′ AMP-activated protein kinase (AMPK) and unc-51-like autophagy activating kinase (ULK1) along with decreased mammalian target of rapamycin (mTOR) levels. A study also investigated BPA levels in IVF patients and revealed that the oocyte retirement rate, oocyte maturation rate and embryo implantation rate significantly decreased with increasing urinary BPA concentrations. The researchers also analyzed how BPA impacted mouse folliculogenesis and discovered that BPA exposure leads to a decrease in GC dilation of the follicles and decreased GC-related hormone concentrations (Lin *et al.* 2021*b*).

Apoptosis

A few studies that have examined the effect of BPA on the KGN immortalized GC line have shown that BPA exposure reduces cell viability (Huang *et al.* 2021, Liu *et al.* 2021). After treatment with different concentrations (30 and 50 μM for 24 h) and analysis with different methods, KGN cell viability was significantly reduced. Exposing KGN cells to higher concentrations of BPA also significantly increased cellular ROS levels and decreased the antioxidant ability of the selected antioxidant enzymes SOD, CAT and GSH ($P < 0.05$). Flow cytometry was used to determine the effect of BPA on KGN cell apoptosis. A lower concentration of BPA (30 μM) had no significant effect on cellular apoptosis, while a higher concentration (50 μM) did. Western blotting was used to assess how BPA exposure influences pro- and antiapoptotic Bax and Bcl2 protein expression. BPA exposure decreased the Bcl2/Bax ratio and significantly induced apoptosis. Apoptosis-inducing caspase 3 protein activity was also measured using flow cytometry. Caspase 3 activity did not significantly increase after BPA exposure. BPA exposure also causes intracellular stress by activating apoptosis signal-regulating kinase 1 (ASK1), c-jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK)

signaling cascades. Phosphorylated stress protein levels were analyzed with western blotting (Liu *et al.* 2021). A similar study on KGN cells was conducted by Huang and colleagues (2021) but with different BPA concentrations (0, 0.1, 1, 10 and 100 μM) and times (12 h and 24 h). They discovered that BPA positively influences KGN cell apoptosis in a concentration- and time-dependent manner in comparison with that in the control group. The effects of BPA exposure on cell apoptosis were stronger after a longer period of exposure (24 h), in addition to a significant change in the protein expression levels of the pro- and anti-apoptosis regulators Bax, Bak, Bcl2 and Mcl-1, as measured with western blotting. This method was also used to test caspase 3 activity, which was significantly increased after exposure to 100 μM BPA. Moreover, BPA exposure also drastically decreased the mitochondrial membrane potential after 24 h of exposure to 100 μM BPA. Phosphorylation of stress response factors, such as extracellular signal-regulated kinases 1/2, p38, JNK, AKT and ASK1, was assessed with western blotting, and a significant increase in the phosphorylation of JNK and ASK1 was found (Huang *et al.* 2021).

BPA effects on human GCs isolated from follicular fluid

The results of different studies have shown that BPA has a negative effect on GCs obtained from healthy ovaries and follicular fluid retrieved by ultrasound-guided aspiration during the IVF procedure similar to that on immortalized GC lines. This is not surprising, as we mentioned before that immortalized GCs largely retain the properties of 'healthy' GCs (Zhang *et al.* 2000). However, GCs from the follicular fluid in the IVF procedure are more difficult to access for research than commercially available cell lines (e.g. COV434 and KGN) and can only be used with the permission of the Medical Ethics Committee and the written informed consent of the patient. As a result, few studies of this kind have been published but, similar to cell line studies, BPA has been shown to negatively affect steroidogenesis and gene expression in GCs. Some of these findings are as follows:

Steroidogenesis

There are few studies on human GCs to date that have examined BPA and steroidogenesis. Pogrmic-Majkic and colleagues (2019) analyzed how BPA affects *STAR* and *CYP19A1* mRNA levels in human cumulus GCs and reported that higher BPA concentrations (100 μM) significantly increased *STAR* mRNA levels after a longer exposure time (48 h), whereas lower doses had no significant effect. In contrast, all examined BPA concentrations decreased the *CYP19A1* mRNA expression. The observed results corresponded with later analyses on progesterone and E2 production; BPA exposure increased progesterone production and decreased E2 levels (Pogrmic-Majkic *et al.* 2019). Slightly contradictory outcomes were observed in a 2016 study in which even lower concentrations of BPA significantly impaired E2 production and progesterone synthesis after downregulating steroidogenesis-related proteins and enzymes (CYP11A1, 3β-HSD and CYP19A1). However, BPA exposure had no effect on StAR expression in this study (Mansur *et al.* 2016). Wang and colleagues compared the BPA effect at lower doses (0.01 to 1 μM for 24 h) on GCs from PCOS and non-PCOS patients with significantly different urine BPA concentrations between the two groups. Aromatase and E2 production was impaired in a concentration-dependent manner in GCs from PCOS patients with higher urine BPA levels but not in GCs from non-PCOS patients. The difference between BPA-related outcomes is probably due to the ovarian hormonal treatment of PCOS patients and the small sample sizes but could suggest that BPA exposure has a role in ovarian dysfunction (Wang *et al.* 2017). Ehrlich and colleagues took a slightly different approach and examined the connection between *CYP19* gene expression in GCs, obtained from IVF pactents, and urinary BPA concentration but found no significant association between the two. However, it is not fully understood how well urine BPA concentration mirrors the BPA concentrations in ovarian cells, so future experiments should focus on different comparison models, perhaps follicular fluid instead of urine (Ehrlich *et al.* 2013).

The results show that *in vitro* BPA exposure can affect human GC steroidogenesis and alter steroid hormone production, mostly in a dose-dependent manner, but further studies are needed to examine how every day, chronic exposure to lower BPA concentrations deregulates human ovarian steroidogenesis. The results of the BPA effect on GCs isolated from the follicular fluid of IVF patients may differ from other studies on GCs because of the previous *in vivo* hormonal treatment of the ovaries, but no major discrepancies were found between studies on human and animal models. To date, there are few studies about how BPA exposure affects GCs. Most of them study BPA exposure in relation to steroid hormones and related genes and/or altered gene expression in GCs. There are no data on BPA-induced oxidative stress in GCs.

Gene expression

Some studies of GCs from follicular fluid from IVF procedures showed that higher concentrations of BPA can significantly affect and upregulate the expression of many GC genes involved in various cell mechanisms, namely, cell cycle progression, cell proliferation, lipid and steroid mechanisms, chromosomal segregation, oogenesis and apoptosis (Mansur *et al.* 2017). BPA dose dependently increases tribbles pseudokinase 3

(*TRIB3*) and insulin-like growth factor binding protein 1 (*IGFBP1*) and attenuates 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*HMGCS1*) expression, which are genes involved in ovarian physiology and folliculogenesis. Gene *TRIB3* is involved in lipid metabolism through PPARG activity and insulin-signaling pathways, while *IGFBP1* gene participates in glucose metabolism, follicular growth, steroidogenesis and ovulation and *HMGCS1* participates in cholesterol synthesis (Prudente *et al.* 2012, Kessler *et al.* 2014). Similar to the KGN cell line, BPA downregulates FSH-induced aromatase expression and E2 secretion by stimulating the expression of *PPARγ*, an aromatase inhibitor (Kwintkiewicz *et al.* 2010, Pogrmic-Majkic *et al.* 2019). Interestingly, there is no linear correlation between urinary BPA levels and aromatase (*CYP19*) expression *in vivo* (Ehrlich *et al.* 2013). BPA also decreases the expression of other genes, such as gap junction protein alpha 1 (*GJA1*) and connexin 43 (*Cx43*), which codes for the gap junction protein connexin, through the estrogen-receptor-dependent signaling pathway and MAPK pathway (Lin *et al.* 2021*a*). BPA exposure also changed the expression of extracellular vesicle miRNAs (EV-miRNAs) and their cellular gene targets but only at high concentrations (200–20,000 ng/mL). The EV-miRNA let-7g-5p was the most deregulated EV-miRNA in a study from 2019 (Rodosthenous *et al.* 2019). let-7g-5p overexpression can disrupt important GC functions and induce apoptosis and autophagy. In contrast, BPA exposure downregulates miR-212-3p, which is normally present in GCs and is regulated by LH. miR-212-3p participates in cell signaling and proliferation and is important for cell viability (Rodosthenous *et al.* 2019).

The negative impacts of BPA on GCs from immortalized cell lines and from follicular fluid from IVF procedures are summarized in Fig. 3.

Negative effects of BPA on animal GCs in vitro and in vivo

More *in vitro* studies of GCs have been performed in mammalian animal species, such as mouse, rat, bovine, ovine and porcine. In animals, GCs are retrieved from the ovaries by different methods, such as needle aspiration and enzymatic degradation of ovarian tissue (Fig. 2). Most of these studies have shown that BPA adversely affects ovarian function and GCs in animals, similar to humans. The results of these papers show that animal GCs are very sensitive and respond to even very low BPA concentrations, such as 10^{-7} to 10^{-5} M (Xu *et al.* 2002, Liang *et al.* 2021). The mechanisms of the negative influence of BPA are very similar to those of human GCs and include cell viability (Bujnakova Mlynarcikova & Scsukova 2018, 2021), steroidogenesis (Xu *et al.* 2002, Mlynarčíková *et al.* 2005, Zhou *et al.* 2008, Samardzija *et al.* 2018, Wu *et al.* 2018, Song *et al.* 2019, Téteau *et al.* 2020, Bujnakova Mlynarcikova & Scsukova 2021), gene expression (Zhou *et al.* 2008, Samardzija *et al.* 2018), oxidative stress/ROS production (Lee *et al.* 2019, Sabry *et al.* 2022) and apoptosis (Liang *et al.* 2021, Xu *et al.* 2002) of GCs. Tables 1 and 2 show results of published *in vitro* studies on human and animal GCs, and it can be seen that comparable concentrations and exposure times were used for BPA exposure; moreover, similar negative effects to those in humans were observed for animal GCs. However, the results of some animal studies showed that differences in the outcome (e.g. cell viability outcomes) regarding the concentrations of BPA could possibly be due to different methods, kits and species (Bujnakova Mlynarcikova & Scsukova 2018, 2021).

The advantage of animal models is that they also allow *in vivo* studies of the effects of BPA, which are

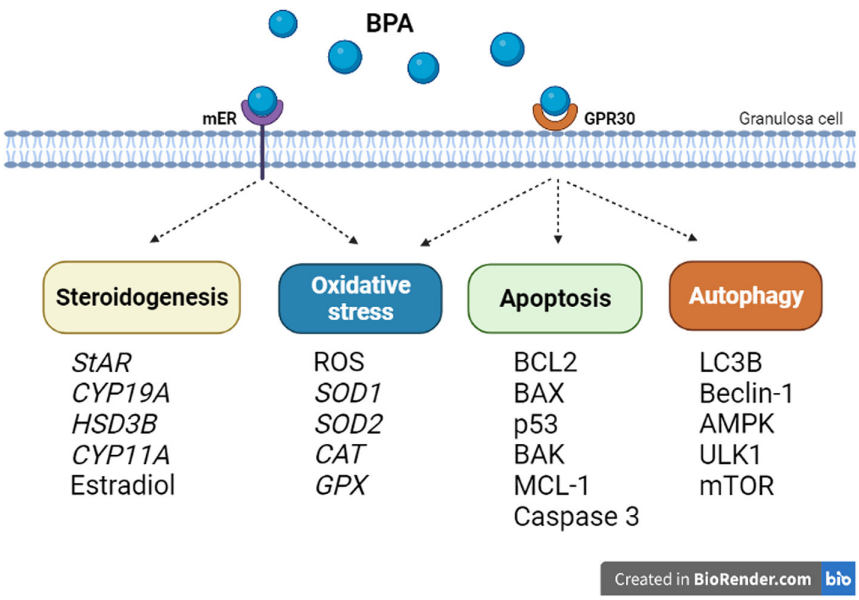

Figure 3 Schematic representation of the GC cellular processes and related genes/proteins that BPA affects. The most researched BPA action is through binding to mER and/or GPR30 receptors, which causes intracellular oxidative stress and increases the expression of genes of oxidative stress pathways. BPA also affects steroidogenesis and alters the synthesis of steroid hormones and expression of associated enzymes. BPA also induces apoptosis by activating pro-apoptotic proteins, a response to BPA-related oxidative stress, and promotes autophagy.

Table 1 Reported negative effects of BPA exposure on the ovarian GCs in human *in vitro* studies.

BPA concentration	Time of exposure	Negative effects on the ovary	Reference
20 µg/mL	48 h	BPA altered genes and involved in cell cycle progression, mitosis, chromosomal segregation, spindle formation, lipid and steroid metabolism.	Mansur *et al.* (2017)
100 µM	48 h	Decreased E2 level and *CYP19A1* mRNA, increased progesterone production, *StAR* and *PPARγ* mRNA expression	Pogrmic-Majkic *et al.* (2019)
2 and 20 µg/mL	48 h	Lowered progesterone biosynthesis, altered E2 production, reduced *3β-HSD, CYP11A1* and *CYP19A1* mRNA levels and 3β-HSD, CYP11A1 and CYP19A1 protein levels	Mansur *et al.* (2017)
10^{-7} M	24 h	Reduced *Cx43* mRNA levels	Lin *et al.* (2020)
1 µM	24 h	Reduced aromatase expression and E2 synthesis	Wang *et al.* (2017)
20, 200, 2000 and 20,000 ng/mL	48 h	Increased levels of let-7g-5p (20, 20,000 ng/mL), miR-191-5p (200 ng/mL) and miR-532 (2000 ng/mL). Decreased levels of miR-125b, miR-212-3p (20 ng/mL), miR-324-5p (200 ng/mL) and miR-27b-3p, miR-335 and miR-572 (20,000 ng/mL)	Rodosthenous *et al.* (2019)
100 µM	48 h	Increased level of *PPARγ* mRNA (100 µM)	Kwinktkiewicz *et al.* (2010)

not possible in humans. *In vivo* studies on animals (mostly murine, porcine, bovine and ovine) use a different approach than experiments on isolated GCs: animals are given a specific dose of BPA and then sacrificed after a specific length of time. GCs are then isolated from ovaries/follicles and analyzed. As such research cannot be performed in humans, these findings are very important, as the effects are assumed to be comparable to those in humans. As in *in vitro* studies, *in vivo* studies have shown that BPA has a negative effect on the ovaries and GCs in mammalian species.

However, GCs from BPA-exposed animals show different responses to BPA treatment. One study reported drastically decreased E2 levels and attenuated P450arom and StAR protein levels in exposed rat GCs. Lower E2 levels were followed by enhanced follicular atresia and luteal regression, probably as a consequence of BPA-induced GC apoptosis (Lee *et al.* 2013). Another study on rat GCs (2019) describes opposite outcomes, with BPA significantly increasing aromatase and E2 expression. Experiments on ovine GCs reported in 2011 and 2015 found no connection between E2 levels and BPA exposure but noted elevated expression of ERα, ERβ and AR with a high cell proliferation rate in BPA-treated GCs. BPA can also alter the GC response to FSH, which can lead to weakened follicular and oocyte development (Rivera *et al.* 2011, 2015).

BPA has also been proven to increase the expression of miRNA-224, a microRNA precursor that is active in mammalian ovaries, specifically GCs. BPA promotes p27 expression in ovine GCs, which results in a previously

Table 2 Reported negative effects of BPA exposure on the ovarian GCs in animal *in vitro* studies.

BPA concentration	Time of exposure	Negative effects on the ovary	Reference
10 µM	24 h	Increased 17β-E2 concentrations, decreased apoptosis and increased proliferation	Song *et al.* (2019)
100 µM	48 h	Increased *STAR, CYP11A1* and *HSD3B1* mRNA levels and STAR protein levels and decreased basal progesterone production	Samardzija *et al.* (2018)
100 µM	24 and 48 h	Decreased cell viability, increased pro-apoptotic BAX protein levels and BPA-induced G2-to-M arrest.	Xu *et al.* (2002)
10^{-4} M	72 h	Decreased progesterone production	Mlynarcikova & Scsukova (2020)
0, 5 and 50 µg/mL	6, 12, 24, 48 and 72h	Reduced cell viability, increased ROS production and increased antioxidant enzymes *SOD1, SOD2, CAT, GPX1* and *GPX4* mRNA levels (50 µg/mL; 12 h)	Sabry *et al.* (2022)
50 and 200 µmol/L	24 h	Increased cell apoptosis by reducing mitochondrial membrane potential (200 µmol/L) and altered BAX, Bcl-2 and p53 protein expression	Liang *et al.* (2021)
100 µM	72 h	Decreased cell viability, reduced basal progesterone production and decreased *StAR, CYP11A1* and *HSD3B* mRNA levels	Bujnakova Mlynarcikova & Scsukova (2018)
10^{-8} M to 10^{-5} M	72 h	Increased basal progesterone levels and decreased E2 production	Mlynarčíková *et al.* (2005)
50, 100 and 200 µM	48 h	Decreased cell viability (200 µM), reduced cell proliferation (50, 100 and 200 µM), reduced progesterone secretion (100 and 200 µM), altered E2 production and decreased *AR* mRNA levels.	Téteau *et al.* (2020)
10^{-7} to 10^{-5} M	48 h	Increased progesterone production up to 10^{-5} M and decreased at 10^{-4} M, decreased E2 levels (10^{-6} to 10^{-4} M) and altered mRNA expression of *P450arom*, *P450scc* and *STAR* (10^{-6} to 10^{-4} M)	Zhou *et al.* (2008)
10 µM	24 and 48 h	Increased E2 synthesis and elevated levels of CYP19A1 and HSD17B proteins.	Wu *et al.* (2018)

mentioned increase in cell proliferation (Rivera *et al.* 2011, 2015).

BPA can affect GCs through receptor binding or can cause oxidative stress via oxidative damage generated by cytotoxic ROS. Banerjee *et al.* studied the possible harmful effects of BPA-generated ROS on GCs isolated from treated rats. BPA-exposed GCs showed increased levels of lipid peroxidation and the inflammation markers MDA and nitric oxide along with increased levels of the proinflammatory cytokines tumor necrosis factor alpha and interleukin, which are consequences of BPA-induced oxidative stress. BPA exposure also markedly depleted antioxidant levels, namely, GSH and SOD, in treated GCs. The results showed decreased CAT activity and expression, which is clearly a response to BPA-induced oxidative damage (Banerjee *et al.* 2018).

Protective role of folic acid

Research on EDs is important not only for a better understanding of the causes and development of infertility, ovarian cancer and other diseases but also for reducing the toxic effects of EDs. Folic acid is an interesting option. In general, folic acid (vitamin B9) is a stable synthetic water-soluble dietary supplement that is converted into biologically active 5-methyltetrahydrofolate through several reactions in the liver. The human body's inability to synthesize it by itself makes folic acid an essential nutrient necessary for cell division and amino acid metabolism (Gliszczynskaswiglo 2007). Folates have an important role as cofactors and coenzymes in the methylation cycle (as a methyl group donor) and DNA/RNA synthesis as donors of one-carbon groups. Folic acid and folates in general are also known for their antioxidant, cardiovascular, anticancer and neuroprotective properties. Folates have been attributed antioxidant activity because they reduce homocysteine concentrations and increase total antioxidant capacity. In this way, they protect biological molecules from oxidative damage induced by free radicals and prevent lipid peroxidation by scavenging and neutralizing highly reactive oxygen radicals. Regular folic acid supplementation (5 mg/day) increases total serum and plasma antioxidant capacity and reduces MDA levels within different researched subgroups (Asbaghi *et al.* 2021).

In the first few studies in women with elevated BPA levels in biological samples, it was found that when taking folic acid, the time required to conceive was shorter than in women who did not take folic acid (Philips *et al.* 2018). It was also found that higher urinary BPA concentrations occurred in the Netherlands in 2004–2005, which was positively associated with female obesity and the lack of folic acid use (Philips *et al.* 2018). Elevated BPA in the urine of patients involved in the IVF program was associated with a minor probability of embryo implantation (66%) in patients receiving <400 μg folate/day as a dietary supplement but not in patients receiving ≥400 μg folate/day (21% more likely to experience embryo implantation); a similar effect has also been identified for the possibility of clinical pregnancy and the birth of a live child (Mínguez-Alarcón *et al.* 2015). Patients with low serum AMH concentrations (poor ovarian reserve) included in the IVF program had higher blood AMH levels and an improved possibility of pregnancy, including naturally achieved pregnancy, after treatment with methyl donors such as folic acid (Silvestris *et al.* 2017). This result indicates the importance of this research, as future findings could be used to improve the chances of conceiving and giving birth in women with higher levels of EDs in biological samples who have difficulty conceiving. In established clinical practice, folic acid is prescribed to pregnant women for healthy fetal development (van Gool *et al.* 2018, Kancherla *et al.* 2022) but not in patients with high concentrations of EDs in their biological samples. In addition, folic acid has been shown to reduce the toxic effects of EDs in animal models (Bilgi *et al.* 2019, Lee *et al.* 2019).

Animal research is very important for a better understanding of the protective role of folic acid, as it can better elucidate its effects. Various animal studies, including in mammals, have shown that the diet or treatment of females with folic acid reduces the toxic effect of BPA on the ovaries, fertility and the development of pups from various aspects: it increases the activity of various antioxidant enzymes (e.g. CAT, glutathione peroxidase, and SOD) and reduces cellular oxidative stress in the blood plasma and liver of females and pups (Mou *et al.* 2018). Folic acid is also thought to prevent the binding of BPA to the centrosome of the dividing spindle and chromosome centromeres, thereby reducing cell division abnormalities and cell aneuploidy (Parry *et al.* 2002). It was suggested relatively early that treatment of female animal models with methyl donors such as folic acid nullifies the negative effect of BPA and other EDs at the epigenetic level (Dolinoy *et al.* 2007). In a study of pigs exposed to BPA during gestation, it was found that this treatment causes intestinal problems (reduced degradation and absorption of food) in pups; however, this negative effect was strongly reduced or eliminated in females receiving a mixture of folic acid, which was associated with methylating DNA in intestinal cells (Liu *et al.* 2017). In an animal model, it has also been found that exposure of females to BPA during gestation causes problems with the pancreas in pups due to hypermethylation of DNA for the insulin-like growth factor 2 (*Igf2)* gene, which did not occur in females receiving folic acid during BPA exposure (Mao *et al.* 2017). In mice, the toxic epigenetic effect of BPA (hypomethylation of different genes) was reduced or eliminated by treating (through feeding) females with folic acid (Singh & Li 2012). *In vivo* animal studies have

shown that female exposure to BPA during gestation is also reflected at the epigenetic level in the offspring, so the protective role of folic acid is even more important.

Conclusions

Until now, there has not been much research investigating the effect of BPA on human granulosa cells and how it alters their functions. These studies include different models: immortal human GC lines, human GCs acquired from IVF patients, and animal GCs *in vitro* or isolated from BPA-treated animals (e.g. murine, porcine and ovine GCs). Most of these studies explore different, specific aspects of BPA exposure but also commonly cover basic methods, such as cell viability, streodiogenesis, gene expressions and so on. Despite this, some of the reported results in animals are contradicting and fail to offer conclusive answers combined possibly due to different species, kits and methods, but most of them confirm the findings in humans. In more articles, GCs were exposed to BPA concentrations different from those relevant in the environment. Despite the negative effects that were observed, the results are not entirely relevant and cannot be applied to the human organism. The results of both human and animal studies show that BPA negatively affects the GCs in terms of cell viability, proliferation, gene expressions, steroidogenesis, oxidative stress, ROS production, apoptosis and autophagy. Negative influences of BPA can also be expressed at the epigenetic level, which is a cause for concern, as they can also be expressed in offspring. Research on EDs such as BPA is not only important for a better understanding of the causes and development of infertility, ovarian cancer and other diseases including impaired ovarian and GC function but also from the point of view of reducing the toxic effects of EDs. An interesting option is folic acid, a methyl donor, which has already been shown to reduce the toxic effects of BPA in both human and animal models. It is important to continue this research in both human and animal models to better understand and reduce the toxic effects of BPA and other EDs in the future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

D C S performed the literature review, wrote this article and designed the table and figures. I V K designed, revised the article, table and figures and approved the final version to be published.

References

Acconcia F, Pallottini V & Marino M 2015 Molecular mechanisms of action of BPA. *Dose-Response* **13** 1559325815610582. (https://doi.org/10.1177/1559325815610582)

Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugiura H, Ohtaki T, Matsumoto H, Uenoyama Y et al. 2007 Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. *Journal of Reproduction and Development* **53** 367–378. (https://doi.org/10.1262/jrd.18146)

Ahsan N, Ullah H, Ullah W & Jahan S 2018 Comparative effects of Bisphenol S and Bisphenol A on the development of female reproductive system in rats; a neonatal exposure study. *Chemosphere* **197** 336–343. (https://doi.org/10.1016/j.chemosphere.2017.12.118)

Almeida S, Raposo A, Almeida-González M & Carrascosa C 2018 Bisphenol A: food Exposure and Impact on Human Health…. *Comprehensive Reviews in Food Science and Food Safety* **17** 1503–1517. (https://doi.org/10.1111/1541-4337.12388)

Alonso-Magdalena P, Ropero AB, Soriano S, García-Arévalo M, Ripoll C, Fuentes E, Quesada I & Nadal Á 2012 Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. *Molecular and Cellular Endocrinology* **355** 201–207. (https://doi.org/10.1016/j.mce.2011.12.012)

Andrianou XD, Gängler S, Piciu A, Charisiadis P, Zira C, Aristidou K, Piciu D, Hauser R & Makris KC 2016 Human exposures to bisphenol A, bisphenol F and chlorinated bisphenol A derivatives and thyroid function. *PLoS One* **11** e0155237. (https://doi.org/10.1371/journal.pone.0155237)

Asbaghi O, Ghanavati M, Ashtary-Larky D, Bagheri R, Rezaei Kelishadi M, Nazarian B, Nordvall M, Wong A, Dutheil F, Suzuki K *et al.* 2021 Effects of folic acid supplementation on oxidative stress markers: a systematic review and meta-analysis of randomized controlled trials. *Antioxidants* **10** 871. (https://doi.org/10.3390/antiox10060871)

Baerwald AR, Adams GP & Pierson RA 2012 Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Human Reproduction Update* **18** 73–91. (https://doi.org/10.1093/humupd/dmr039)

Banerjee O, Singh S, Prasad SK, Bhattacharjee A, Banerjee A, Banerjee A, Saha A, Maji BK & Mukherjee S 2018 Inhibition of catalase activity with 3-amino-1,2,4-triazole intensifies bisphenol A (BPA)-induced toxicity in granulosa cells of female albino rats. *Toxicology and Industrial Health* **34** 787–797. (https://doi.org/10.1177/0748233718795744)

Barré-Sinoussi F & Montagutelli X 2015 Animal models are essential to biological research: issues and perspectives. *Future Science OA* **1** FSO63. (https://doi.org/10.4155/fso.15.63)

Bedenk J, Vrtačnik-Bokal E & Virant-Klun I 2020 The role of anti-Müllerian hormone (AMH) in ovarian disease and infertility. *Journal of Assisted Reproduction and Genetics* **37** 89–100. (https://doi.org/10.1007/s10815-019-01622-7)

Bellavia A, Zou R, Björvang RD, Roos K, Sjunnesson Y, Hallberg I, Holte J, Pikki A, Lenters V, Portengen L *et al*. 2023 Association between chemical mixtures and female fertility in women undergoing assisted reproduction in Sweden and Estonia. *Environmental Research* **216**(Pt 1) 114447. (https://doi.org/10.1016/j.envres.2022.114447)

Beschta S, Eubler K, Bohne N, Forne I, Berg D, Berg U & Mayerhofer A 2021 A rapid and robust method for the cryopreservation of human granulosa cells. *Histochemistry and Cell Biology* **156** 509–517. (https://doi.org/10.1007/s00418-021-02019-3)

Bilgi A, Abalı R, Bilgi PT, Şahin M, Tunçdemir M & Boran AB 2019 The apoptotic effects of bisphenol A exposure on the rat ovary: an experimental study. *Environmental Science and Pollution Research International* **26** 10198–10203. (https://doi.org/10.1007/s11356-019-04487-6)

Bloom MS, Vom Saal FS, Kim D, Taylor JA, Lamb JD & Fujimoto VY 2011 Serum unconjugated bisphenol A concentrations in men may influence embryo quality indicators during in vitro fertilization. *Environmental Toxicology and Pharmacology* **32** 319–323. (https://doi.org/10.1016/j.etap.2011.06.003)

Bonde JP, Flachs EM, Rimborg S, Glazer CH, Giwercman A, Ramlau-Hansen CH, Hougaard KS, Høyer BB, Hærvig KK, Petersen SB *et al.* 2016 The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. *Human Reproduction Update* **23** 104–125. (https://doi.org/10.1093/humupd/dmw036)

Bousoumah R, Leso V, Iavicoli I, Huuskonen P, Viegas S, Porras SP, Santonen T, Frery N, Robert A & Ndaw S 2021 Biomonitoring of occupational exposure to bisphenol A, bisphenol S and bisphenol F: a systematic review. *Science of the Total Environment* **783** 146905. (https://doi.org/10.1016/j.scitotenv.2021.146905)

Bremer AA & Miller WL 2014 Chapter 13: Regulation of steroidogenesis. In A Ulloa-Aguirre & PM Conn, Eds., *Cellular Endocrinology in Health and Disease*. Boston: Academic Press, pp. 207–227.

Bujnakova Mlynarcikova A & Scsukova S 2018 Simultaneous effects of endocrine disruptor bisphenol A and flavonoid fisetin on progesterone production by granulosa cells. *Environmental Toxicology and Pharmacology* **59** 66–73. (https://doi.org/10.1016/j.etap.2018.03.001)

Bujnakova Mlynarcikova A & Scsukova S 2021 Bisphenol analogs AF and S: effects on cell status and production of angiogenesis-related factors by COV434 human granulosa cell line. *Toxicology and Applied Pharmacology* **426** 115634. (https://doi.org/10.1016/j.taap.2021.115634)

Chen ZJ, Yang XL, Liu H, Wei W, Zhang KS, Huang HB, Giesy JP, Liu HL, Du J & Wang HS 2015 Bisphenol A modulates colorectal cancer protein profile and promotes the metastasis via induction of epithelial to mesenchymal transitions. *Archives of Toxicology* **89** 1371–1381. (https://doi.org/10.1007/s00204-014-1301-z)

Chen D, Kannan K, Tan H, Zheng Z, Feng YL, Wu Y & Widelka M 2016 Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity-A review. *Environmental Science and Technology* **50** 5438–5453. (https://doi.org/10.1021/acs.est.5b05387)

Chen Y, Wang Y, Ding G, Tian Y, Zhou Z, Wang X, Shen L & Huang H 2018 Association between bisphenol a exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. *Environment International* **115** 410-416. (https://doi.org/10.1016/j.envint.2018.02.041)

Chevalier N & Fénichel P 2015 Bisphenol A: Targeting metabolic tissues. *Reviews in Endocrine and Metabolic Disorders* **16** 299–309. (https://doi.org/10.1007/s11154-016-9333-8)

Cimmino I, Fiory F, Perruolo G, Miele C, Beguinot F, Formisano P & Oriente F 2020 Potential mechanisms of bisphenol A (BPA) contributing to human disease. *International Journal of Molecular Sciences* **21** 5761. (https://doi.org/10.3390/ijms21165761)

Crain DA, Janssen SJ, Edwards TM, Heindel J, Ho SM, Hunt P, Iguchi T, Juul A, McLachlan JA, Schwartz J *et al.* 2008 Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. *Fertility and Sterility* **90** 911–940. (https://doi.org/10.1016/j.fertnstert.2008.08.067)

Czubacka E, Wielgomas B, Klimowska A, Radwan M, Radwan P, Karwacka A, Kałużny P & Jurewicz J 2021 Urinary Bisphenol A Concentrations and Parameters of Ovarian Reserve among Women from a Fertility Clinic. *International Journal of Environmental Research and Public Health* **18** 8041. (https://doi.org/10.3390/ijerph18158041)

Ćwiek-Ludwicka K 2015 Bisphenol A (BPA) in food contact materials - new scientific opinion from EFSA regarding public health risk. *Roczniki Panstwowego Zakladu Higieny* **66** 299–307.

Dolinoy DC, Huang D & Jirtle RL 2007 Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proceedings of the National Academy of Sciences of the United States of America* **104** 13056–13061. (https://doi.org/10.1073/pnas.0703739104)

Ehrlich S, Williams PL, Hauser R, Missmer SA, Peretz J, Calafat AM & Flaws JA 2013 Urinary bisphenol A concentrations and cytochrome P450 19 A1 (Cyp19) gene expression in ovarian granulosa cells: an in vivo human study. *Reproductive Toxicology* **42** 18–23. (https://doi.org/10.1016/j.reprotox.2013.06.071)

Fang H, Tong W, Perkins R, Soto AM, Prechtl NV & Sheehan DM 2000 Quantitative comparisons of in vitro assays for estrogenic activities. *Environmental Health Perspectives* **108** 723–729. (https://doi.org/10.1289/ehp.00108723)

Farrugia F, Aquilina A, Vassallo J & Pace NP 2021 Bisphenol A and type 2 diabetes mellitus: a review of epidemiologic, functional, and early life factors. *International Journal of Environmental Research and Public Health* **18** E716. (https://doi.org/10.3390/ijerph18020716)

Fortune JE, Cushman RA, Wahl CM & Kito S 2000 The primordial to primary follicle transition. *Molecular and Cellular Endocrinology* **163** 53–60. (https://doi.org/10.1016/s0303-7207(99)00240-3)

Gallo A 2022 Reprotoxic impact of environment, diet, and behavior. *International Journal of Environmental Research and Public Health* **19** 1303. (https://doi.org/10.3390/ijerph19031303)

Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, Rubio S, Monfort N, Ventura R, Martínez D *et al.* 2015 Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. *Journal of Allergy and Clinical Immunology* **135** 370–378. (https://doi.org/10.1016/j.jaci.2014.09.030)

Gassman NR 2017 Induction of oxidative stress by bisphenol A and its pleiotropic effects. *Environmental and Molecular Mutagenesis* **58** 60–71. (https://doi.org/10.1002/em.22072)

Geens T, Aerts D, Berthot C, Bourguignon JP, Goeyens L, Lecomte P, Maghuin-Rogister G, Pironnet AM, Pussemier L, Scippo ML *et al.* 2012 A review of dietary and non-dietary exposure to bisphenol-A. *Food and Chemical Toxicology* **50** 3725–3740. (https://doi.org/10.1016/j.fct.2012.07.059)

Gliszczynskaswiglo A 2007 Folates as antioxidants. *Food Chemistry* **101** 1480–1483. (https://doi.org/10.1016/j.foodchem.2006.04.022)

Gore AC, Crews D, Doan LL, Merrill ML, Patisaul H & Zota A 2014 *Introduction to Endocrine Disrupting Chemicals (EDCs); A Guide for Public Interest Organizations and Policy-Makers*. Washington, DC: Endocrine Society.

Gorini F, Bustaffa E, Coi A, Iervasi G & Bianchi F 2020 Bisphenols as environmental triggers of thyroid dysfunction: clues and evidence. *International Journal of Environmental Research and Public Health* **17** 2654. (https://doi.org/10.3390/ijerph17082654)

Guo Y, Li B, Yan X, Shen X, Ma J, Liu S & Zhang D 2020 Bisphenol A and polychlorinated biphenyls enhance the cancer stem cell properties of human ovarian cancer cells by activating the Wnt signaling pathway. *Chemosphere* **246** 125775. (https://doi.org/10.1016/j.chemosphere.2019.125775)

Hartle JC, Zawadzki RS, Rigdon J, Lam J & Gardner CD 2022 Development and evaluation of a novel dietary bisphenol A (BPA) exposure risk tool. *BMC Nutrition* **8** 143. (https://doi.org/10.1186/s40795-022-00634-4)

Hatzirodos N, Hummitzsch K, Irving-Rodgers HF & Rodgers RJ 2015 Transcriptome comparisons identify new cell markers for theca interna and granulosa cells from small and large antral ovarian follicles. *PLoS One* **10** e0119800. (https://doi.org/10.1371/journal.pone.0119800)

Heindel JJ, Blumberg B, Cave M, Machtinger R, Mantovani A, Mendez MA, Nadal A, Palanza P, Panzica G, Sargis R *et al.* 2017 Metabolism disrupting chemicals and metabolic disorders. *Reproductive Toxicology* **68** 3–33. (https://doi.org/10.1016/j.reprotox.2016.10.001)

Hines CJ, Jackson MV, Deddens JA, Clark JC, Ye X, Christianson AL, Meadows JW & Calafat AM 2017 Urinary bisphenol A (BPA) concentrations among workers in industries that manufacture and use BPA in the USA. *Annals of Work Exposures and Health* **61** 164–182. (https://doi.org/10.1093/annweh/wxw021)

Hoffmann M, Gogola J, Kotula-Balak M & Ptak A 2017 Stimulation of ovarian cell proliferation by tetrabromobisphenol A but not tetrachlorobisphenol A through G protein-coupled receptor 30. *Toxicology in Vitro* **45** 54–59. (https://doi.org/10.1016/j.tiv.2017.08.009)

Hoffmann M, Rak A & Ptak A 2018 Bisphenol A and its derivatives decrease expression of chemerin, which reverses its stimulatory action in ovarian cancer cells. *Toxicology Letters* **291** 61–69. (https://doi.org/10.1016/j.toxlet.2018.04.004)

Huang R-P, Liu Z-H, Yuan S-F, Yin H, Dang Z & Wu P-X 2017 Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000-2016) and its risk analysis. *Environmental Pollution* **230** 143–152. (https://doi.org/10.1016/j.envpol.2017.06.026)

Huang RP, Liu ZH, Yin H, Dang Z, Wu PX, Zhu NW & Lin Z 2018 Bisphenol A concentrations in human urine, human intakes across

six continents, and annual trends of average intakes in adult and child populations worldwide: a thorough literature review. *Science of the Total Environment* **626** 971–981. (https://doi.org/10.1016/j.scitotenv.2018.01.144)

Huang M, Liu S, Fu L, Jiang X & Yang M 2020 Bisphenol A and its analogues bisphenol S, bisphenol F and bisphenol AF induce oxidative stress and biomacromolecular damage in human granulosa KGN cells. *Chemosphere* **253** 126707. (https://doi.org/10.1016/j.chemosphere.2020.126707)

Huang M, Huang M, Li X, Liu S, Fu L, Jiang X & Yang M 2021 Bisphenol A induces apoptosis through GPER-dependent activation of the ROS/Ca2+-ASK1-JNK pathway in human granulosa cell line KGN. *Ecotoxicology and Environmental Safety* **208** 111429. (https://doi.org/10.1016/j.ecoenv.2020.111429)

Jones RE & Lopez KH 2006 *Human Reproductive Biology*. 3rd Edition. Elsevier Science. (https://doi.org/10.1016/C2009-0-02024-8)

Jozkowiak M, Piotrowska-Kempisty H, Kobylarek D, Gorska N, Mozdziak P, Kempisty B, Rachon D & Spaczynski RZ 2022 Endocrine Disrupting Chemicals in Polycystic Ovary Syndrome: The Relevant Role of the Theca and Granulosa Cells in the Pathogenesis of the Ovarian Dysfunction. *Cells* **12** 174. (https://doi.org/10.3390/cells12010174)

Jun JH, Oh JE, Shim JK, Kwak YL & Cho JS 2021 Effects of bisphenol A on the proliferation, migration, and tumor growth of colon cancer cells: in vitro and in vivo evaluation with mechanistic insights related to ERK and 5-HT3. *Food and Chemical Toxicology* **158** 112662. (https://doi.org/10.1016/j.fct.2021.112662)

Kabir ER, Rahman MS & Rahman I 2015 A review on endocrine disruptors and their possible impacts on human health. *Environmental Toxicology and Pharmacology* **40** 241–258. (https://doi.org/10.1016/j.etap.2015.06.009)

Kancherla V, Wagh K, Priyadarshini P, Pachón H & Oakley GP 2022 A global update on the status of prevention of folic acid-preventable spina bifida and anencephaly in year 2020: 30-Year anniversary of gaining knowledge about folic acid's prevention potential for neural tube defects. *Birth Defects Research* **114** 1392–1403. (https://doi.org/10.1002/bdr2.2115)

Kechagias KS, Semertzidou A, Athanasiou A, Paraskevaidi M & Kyrgiou M 2020 Bisphenol-A and polycystic ovary syndrome: a review of the literature. *Reviews on Environmental Health* **35** 323–331. (https://doi.org/10.1515/reveh-2020-0032)

Kessler EC, Gross JJ, Bruckmaier RM & Albrecht C 2014 Cholesterol metabolism, transport, and hepatic regulation in dairy cows during transition and early lactation. *Journal of Dairy Science* **97** 5481–5490. (https://doi.org/10.3168/jds.2014-7926)

Kiess W, Häussler G & Vogel M 2021 Endocrine-disrupting chemicals and child health. *Best Practice and Research in Clinical Endocrinology and Metabolism* **35** 101516. (https://doi.org/10.1016/j.beem.2021.101516)

Kim MJ & Park YJ 2019 Bisphenols and thyroid hormone. *Endocrinology and Metabolism* **34** 340–348. (https://doi.org/10.3803/EnM.2019.34.4.340)

Konieczna A, Rutkowska A & Rachoń D 2015 Health risk of exposure to bisphenol A (BPA). *Roczniki Panstwowego Zakladu Higieny* **66** 5–11.

Kossowska-Tomaszczuk K, De Geyter C, De Geyter M, Martin I, Holzgreve W, Scherberich A & Zhang H 2009 The multipotency of luteinizing granulosa cells collected from mature ovarian follicles. *Stem Cells* **27** 210–219. (https://doi.org/10.1634/stemcells.2008-0233)

Kouidhi W, Thannimalay L, Soon CS & Ali Mohd M 2017 Occupational exposure to bisphenol A (BPA) in a plastic injection molding factory in Malaysia. *International Journal of Occupational Medicine and Environmental Health* **30** 743–750. (https://doi.org/10.13075/ijomeh.1896.00917)

Kwintkiewicz J, Nishi Y, Yanase T & Giudice LC 2010 Peroxisome proliferator–activated receptor-γ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environmental Health Perspectives* **118** 400–406. (https://doi.org/10.1289/ehp.0901161)

Lagarde F, Beausoleil C, Belcher SM, Belzunces LP, Emond C, Guerbet M & Rousselle C 2015 Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. *Environmental Health: a Global Access Science Source* **14** 13. (https://doi.org/10.1186/1476-069X-14-13)

Land KL, Miller FG, Fugate AC & Hannon PR 2022 The effects of endocrine-disrupting chemicals on ovarian- and ovulation-related fertility outcomes. *Molecular Reproduction and Development* **89** 608-631. (https://doi.org/10.1002/mrd.23652)

Lazúrová Z, Figurová J, Hubková B, Mašlanková J & Lazúrová I 2021 Urinary bisphenol A in women with polycystic ovary syndrome - a possible suppressive effect on steroidogenesis? *Hormone Molecular Biology and Clinical Investigation* **42** 303-309. (https://doi.org/10.1515/hmbci-2020-0032)

Lee HJ, Chattopadhyay S, Gong E-Y, Ahn RS & Lee K 2003 Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicological Sciences* **75** 40–46. (https://doi.org/10.1093/toxsci/kfg150)

Lee SG, Kim JY, Chung JY, Kim YJ, Park JE, Oh S, Yoon YD, Yoo KS, Yoo YH & Kim JM 2013 Bisphenol A exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17β-estradiol synthesis via downregulation of aromatase in rat ovary. *Environmental Health Perspectives* **121** 663–669. (https://doi.org/10.1289/ehp.1205823)

Lee CT, Wang JY, Chou KY & Hsu MI 2019 1,25-Dihydroxyvitamin D3 modulates the effects of sublethal BPA on mitochondrial function via activating PI3K-Akt pathway and 17β-estradiol secretion in rat granulosa cells. *Journal of Steroid Biochemistry and Molecular Biology* **185** 200–211. (https://doi.org/10.1016/j.jsbmb.2018.09.002)

Li C, Cao M, Qi T, Ye X, Ma L, Pan W, Luo J, Chen P, Liu J & Zhou J 2021 The association of bisphenol A exposure with premature ovarian insufficiency: a case-control study. *Climacteric* **24** 95-100. (https://doi.org/10.1080/13697137.2020.1781078)

Liang M, Zhou J, Sun X, He C, Zhang K & Hu K 2021 Effects of bisphenol A on apoptosis of ovarian preantral follicular granulosa cells and ovarian development in mice. *Nan Fang Yi Ke Da Xue Xue Bao=Journal of Southern Medical University* **41** 93–99. (https://doi.org/10.12122/j.issn.1673-4254.2021.01.13)

Lin Y, Qiu X, Liu J, Tseng CH, Allard P, Araujo JA & Zhu Y 2020 Different temporal trends of exposure to bisphenol A among international travelers between los Angeles and Beijing. *Environment International* **141** 105758. (https://doi.org/10.1016/j.envint.2020.105758)

Lin M, Hua R, Ma J, Zhou Y, Li P, Xu X, Yu Z & Quan S 2021*a* Bisphenol A promotes autophagy in ovarian granulosa cells by inducing AMPK/mTOR/ULK1 signalling pathway. *Environment International* **147** 106298. (https://doi.org/10.1016/j.envint.2020.106298)

Lin TC, Wang KH, Chuang KH, Kao AP & Kuo TC 2021*b* Downregulation of gap junctional intercellular communication and connexin 43 expression by bisphenol A in human granulosa cells. *Biotechnology and Applied Biochemistry* **68** 676–682. (https://doi.org/10.1002/bab.1979)

Liu H, Wang J, Mou D, Che L, Fang Z, Feng B, Lin Y, Xu S, Li J & Wu D 2017 Maternal Methyl Donor Supplementation during Gestation Counteracts the Bisphenol A-Induced Impairment of Intestinal Morphology, Disaccharidase Activity, and Nutrient Transporters Gene Expression in Newborn and Weaning Pigs. *Nutrients* **9** 423. (https://doi.org/10.3390/nu9050423)

Liu J, Wattar N, Field CJ, Dinu I, Dewey D, Martin JW & **APrON study team** 2018 Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. *Environment International* **119** 319–326. (https://doi.org/10.1016/j.envint.2018.07.001)

Liu S, Xu G, Huang M, Fu L, Jiang X & Yang M 2021*a* Bisphenol A and bisphenol AF co-exposure induces apoptosis in human granulosa cell line KGN through intracellular stress-dependent mechanisms. *Arabian Journal of Chemistry* **14** 103399. (https://doi.org/10.1016/j.arabjc.2021.103399)

Liu H, Jin H, Kim G & Bae J 2021*b* A low dose of bisphenol A stimulates estradiol production by regulating β-catenin-FOXL2-CYP19A1 pathway in human ovarian granulosa cells. *Biochemical and Biophysical Research Communications* **583** 192–198. (https://doi.org/10.1016/j.bbrc.2021.10.070)

Ma S, Shi W, Wang X, Song P & Zhong X 2017 Bisphenol A Exposure during Pregnancy Alters the Mortality and Levels of Reproductive Hormones and Genes in Offspring Mice. *BioMed Research International* **2017** 3585809. (https://doi.org/10.1155/2017/3585809)

Ma Y, Liu H, Wu J, Yuan L, Wang Y, Du X, Wang R, Marwa PW, Petlulu P, Chen X *et al*. 2019 The adverse health effects of bisphenol A and related toxicity mechanisms. *Environmental Research* **176** 108575. (https://doi.org/10.1016/j.envres.2019.108575)

Maffini MV, Rubin BS, Sonnenschein C & Soto AM 2006 Endocrine disruptors and reproductive health: the case of bisphenol-A. *Molecular and Cellular Endocrinology* **254–255** 179–186. (https://doi.org/10.1016/j.mce.2006.04.033)

Mansur A, Adir M, Yerushalmi G, Hourvitz A, Gitman H, Yung Y, Orvieto R & Machtinger R 2016 Does BPA alter steroid hormone synthesis in human granulosa cells in vitro? *Human Reproduction* **31** 1562–1569. (https://doi.org/10.1093/humrep/dew088)

Mansur A, Israel A, Combelles CMH, Adir M, Racowsky C, Hauser R, Baccarelli AA & Machtinger R 2017 Bisphenol-A exposure and gene expression in human luteinized membrana granulosa cells in vitro. *Human Reproduction* **32** 409–417. (https://doi.org/10.1093/humrep/dew316)

Mao B, Qiu J, Zhao N, Shao Y, Dai W, He X, Cui H, Lin X, Lv L, Tang Z et al. 2017 Maternal folic acid supplementation and dietary folate intake and congenital heart defects. *PloS One* **12** e0187996. (https://doi.org/10.1371/journal.pone.0187996)

Marino M, Pellegrini M, La Rosa P & Acconcia F 2012 Susceptibility of estrogen receptor rapid responses to xenoestrogens: physiological outcomes. *Steroids* **77** 910–917. (https://doi.org/10.1016/j.steroids.2012.02.019)

Marti N, Galván JA, Pandey AV, Trippel M, Tapia C, Müller M, Perren A & Flück CE 2017 Genes and proteins of the alternative steroid backdoor pathway for dihydrotestosterone synthesis are expressed in the human ovary and seem enhanced in the polycystic ovary syndrome. *Molecular and Cellular Endocrinology* **441** 116–123. (https://doi.org/10.1016/j.mce.2016.07.029)

Meli R, Monnolo A, Annunziata C, Pirozzi C & Ferrante MC 2020 Oxidative stress and BPA toxicity: an antioxidant approach for male and female reproductive dysfunction. *Antioxidants (Basel, Switzerland)* **9** E405. (https://doi.org/10.3390/antiox9050405)

Miao M, Yuan W, Yang F, Liang H, Zhou Z, Li R, Gao E & Li D-K 2015 Associations between Bisphenol A Exposure and Reproductive Hormones among Female Workers. *International Journal of Environmental Research and Public Health* **12** 13240–13250. (https://doi.org/10.3390/ijerph121013240)

Miller WL & Auchus RJ 2011 The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine Reviews* **32** 81–151. (https://doi.org/10.1210/er.2010-0013)

Mínguez-Alarcón L, Gaskins AJ, Chiu YH, Williams PL, Ehrlich S, Chavarro JE, Petrozza JC, Ford JB, Calafat AM, Hauser R *et al.* 2015 Urinary bisphenol A concentrations and association with in vitro fertilization outcomes among women from a fertility clinic. *Human Reproduction* **30** 2120–2128. (https://doi.org/10.1093/humrep/dev183)

Mlynarcikova AB & Scsukova S 2020 Effect of selected bisphenol derivatives on nuclear receptor expression in ovarian cell line COV434. *Endocrine Regulations* **54** 275–283. (https://doi.org/10.2478/enr-2020-0031)

Mlynarčíková A, Kolena J, Ficková M & Scsuková S 2005 Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate. *Molecular and Cellular Endocrinology* **244** 57–62. (https://doi.org/10.1016/j.mce.2005.02.009)

Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X & Hauser R 2010 Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. *International Journal of Andrology* **33** 385–393. (https://doi.org/10.1111/j.1365-2605.2009.01014.x)

Monniaux D, Cadoret V, Clément F, Dalbies-Tran R, Elis S, Fabre S, Maillard V, Monget P & Uzbekova S 2019 Folliculogenesis. In I Huhtaniemi & L Martini, Eds. *Encyclopedia of Endocrine Diseases*, 2nd ed. Oxford: Academic Press, pp. 377–398. (https://doi.org/10.1016/B978-0-12-812199-3.64550-4)

Moon MK, Kim MJ, Jung IK, Koo YD, Ann HY, Lee KJ, Kim SH, Yoon YC, Cho BJ, Park KS *et al.* 2012 Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. *Journal of Korean Medical Science* **27** 644–652. (https://doi.org/10.3346/jkms.2012.27.6.644)

Moreno-Gómez-Toledano R, Arenas MI, Vélez-Vélez E, Saura M & Bosch RJ 2021 New evidence of renal and cardiovascular alterations promoted by bisphenol A. *Biomolecules* **11** 1649. (https://doi.org/10.3390/biom11111649)

Mou D, Wang J, Liu H, Chen Y, Che L, Fang Z, Xu S, Lin Y, Feng B, Li J et al. 2018 Maternal methyl donor supplementation during gestation counteracts bisphenol A-induced oxidative stress in sows and offspring. *Nutrition* **45** 76–84. (https://doi.org/10.1016/j.nut.2017.03.012)

Moustafa GG & Ahmed AAM 2016 Impact of prenatal and postnatal exposure to bisphenol A on female rats in a two generational study: Genotoxic and immunohistochemical implications. *Toxicology Reports* **3** 685-695. (https://doi.org/10.1016/j.toxrep.2016.08.008)

Mukhopadhyay R, Prabhu NB, Kabekkodu SP & Rai PS 2022 Review on bisphenol A and the risk of polycystic ovarian syndrome: an insight from endocrine and gene expression. *Environmental Science and Pollution Research International* **29** 32631-32650. (https://doi.org/10.1007/s11356-022-19244-5)

Nadal A, Fuentes E, Ripoll C, Villar-Pazos S, Castellano-Muñoz M, Soriano S, Martinez-Pinna J, Quesada I & Alonso-Magdalena P 2018 Extranuclear-initiated estrogenic actions of endocrine disrupting chemicals: Is there toxicology beyond paracelsus? *The Journal of Steroid Biochemistry and Molecular Biology* **176** 16–22. (https://doi.org/10.1016/j.jsbmb.2017.01.014)

Naomi R, Yazid MD, Bahari H, Keong YY, Rajandram R, Embong H, Teoh SH, Halim S & Othman F 2022 Bisphenol A (BPA) leading to obesity and cardiovascular complications: a compilation of current in vivo study. *International Journal of Molecular Sciences* **23** 2969. (https://doi.org/10.3390/ijms23062969)

Nishi Y, Yanase T, Mu Y, Oba K, Ichino I, Saito M, Nomura M, Mukasa C, Okabe T, Goto K *et al.* 2001 Establishment and characterization of a steroidogenic human granulosa-like tumor cell line, KGN, that expresses functional follicle-stimulating hormone receptor. *Endocrinology* **142** 437–445. (https://doi.org/10.1210/endo.142.1.7862)

Oliveira IM, Romano RM, de Campos P, Cavallin MD, Oliveira CA & Romano MA 2017 Delayed onset of puberty in male offspring from bisphenol A-treated dams is followed by the modulation of gene expression in the hypothalamic-pituitary-testis axis in adulthood. *Reproduction, Fertility and Development* **29** 2496-2505. (https://doi.org/10.1071/RD17107)

Parry EM, Parry JM, Corso C, Doherty A, Haddad F, Hermine TF, Johnson G, Kayani M, Quick E, Warr T et al. 2002 Detection and characterization of mechanisms of action of aneugenic chemicals. *Mutagenesis* **17** 509–521. (https://doi.org/10.1093/mutage/17.6.509)

Perera F, Nolte ELR, Wang Y, Margolis AE, Calafat AM, Wang S, Garcia W, Hoepner LA, Peterson BS, Rauh V *et al.* 2016 Bisphenol A exposure and symptoms of anxiety and depression among inner city children at 10–12 years of age. *Environmental Research* **151** 195–202. (https://doi.org/10.1016/j.envres.2016.07.028)

Peretz J, Craig ZR & Flaws JA 2012 Bisphenol A inhibits follicle growth and induces atresia in cultured mouse antral follicles independently of the genomic estrogenic Pathway1. *Biology of Reproduction* **87** 1–11. (https://doi.org/10.1095/biolreprod.112.101899)

Philips EM, Kahn LG, Jaddoe VWV, Shao Y, Asimakopoulos AG, Kannan K, Steegers EAP & Trasande L 2018 First Trimester Urinary Bisphenol and Phthalate Concentrations and Time to Pregnancy: A Population-Based Cohort Analysis. *The Journal of Clinical Endocrinology and Metabolism* **103** 3540–3547. (https://doi.org/10.1210/jc.2018-00855)

Pivonello C, Muscogiuri G, Nardone A, Garifalos F, Provvisiero DP, Verde N, de Angelis C, Conforti A, Piscopo M, Auriemma RS *et al.* 2020 Bisphenol A: an emerging threat to female fertility. *Reproductive Biology and Endocrinology: RB&E* **18** 22. (https://doi.org/10.1186/s12958-019-0558-8)

Pogrmic-Majkic K, Samardzija Nenadov D, Fa S, Stanic B, Trninic Pjevic A & Andric N 2019 BPA activates EGFR and ERK1/2 through PPARγ to increase expression of steroidogenic acute regulatory protein in human cumulus granulosa cells. *Chemosphere* **229** 60–67. (https://doi.org/10.1016/j.chemosphere.2019.04.174)

Prudente S, Sesti G, Pandolfi A, Andreozzi F, Consoli A & Trischitta V 2012 The mammalian tribbles homolog TRIB3, glucose homeostasis, and cardiovascular diseases. *Endocrine Reviews* **33** 526–546. (https://doi.org/10.1210/er.2011-1042)

Qi J, Liu L, Yang J, Gao X & Zhang W 2020 Bisphenol A decreases progesterone synthesis in human ovarian granulosa cells. *Birth Defects Research* **112** 1843–1849. (https://doi.org/10.1002/bdr2.1817)

Radwan M, Wielgomas B, Dziewirska E, Radwan P, Kałużny P, Klimowska A, Hanke W & Jurewicz J 2018 Urinary bisphenol A levels and male fertility. *American Journal of Men's Health* **12** 2144–2151. (https://doi.org/10.1177/1557988318799163)

Rajkumar A, Luu T, Beal MA, Barton-Maclaren TS, Robaire B & Hales BF 2021 Elucidation of the effects of bisphenol A and structural analogs on germ and steroidogenic cells using single cell high-content imaging. *Toxicological Sciences* **180** 224–238. (https://doi.org/10.1093/toxsci/kfab012)

Rashid H, Alqahtani SS & Alshahrani S 2020 Diet: a source of endocrine disruptors. *Endocrine, Metabolic and Immune Disorders Drug Targets* **20** 633–645. (https://doi.org/10.2174/1871530319666191022100141)

Rattan S, Zhou C, Chiang C, Mahalingam S, Brehm E & Flaws JA 2017 Exposure to endocrine disruptors during adulthood: consequences for female fertility. *Journal of Endocrinology* **233** R109–R129. (https://doi.org/10.1530/JOE-17-0023)

Rebuli ME, Cao J, Sluzas E, Delclos KB, Camacho L, Lewis SM, Vanlandingham MM & Patisaul HB 2014 Investigation of the effects of subchronic low dose oral exposure to bisphenol A (BPA) and ethinyl estradiol (EE) on estrogen receptor expression in the juvenile and adult female rat hypothalamus. *Toxicological Sciences* **140** 190-203. (https://doi.org/10.1093/toxsci/kfu074)

Rezg R, El-Fazaa S, Gharbi N & Mornagui B 2014 Bisphenol A and human chronic diseases: current evidences, possible mechanisms, and future perspectives. *Environment International* **64** 83–90. (https://doi.org/10.1016/j.envint.2013.12.007)

Ribeiro E, Ladeira C & Viegas S 2017 Occupational exposure to bisphenol A (BPA): a reality that still needs to be unveiled. *Toxics* **5** 22. (https://doi.org/10.3390/toxics5030022)

Rivera OE, Varayoud J, Rodríguez HA, Muñoz-de-Toro M & Luque EH 2011 Neonatal exposure to bisphenol A or diethylstilbestrol alters the ovarian follicular dynamics in the lamb. *Reproductive Toxicology* **32** 304–312. (https://doi.org/10.1016/j.reprotox.2011.06.118)

Rivera OE, Varayoud J, Rodríguez HA, Santamaría CG, Bosquiazzo VL, Osti M, Belmonte NM, Muñoz-de-Toro M & Luque EH 2015 Neonatal exposure to xenoestrogens impairs the ovarian response to gonadotropin treatment in lambs. *Reproduction* **149** 645–655. (https://doi.org/10.1530/REP-14-0567)

Rochester JR & Bolden AL 2015 Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. *Environmental Health Perspectives* **123** 643–650. (https://doi.org/10.1289/ehp.1408989)

Rodosthenous RS, Baccarelli AA, Mansour A, Adir M, Israel A, Racowsky C, Hauser R, Bollati V & Machtinger R 2019 Supraphysiological concentrations of bisphenol A alter the expression of extracellular vesicle-enriched miRNAs from human primary granulosa cells. *Toxicological Sciences* **169** 5–13. (https://doi.org/10.1093/toxsci/kfz020)

Rutkowska A & Rachoń D 2014 Bisphenol A (BPA) and its potential role in the pathogenesis of the polycystic ovary syndrome (PCOS). *Gynecological Endocrinology* **30** 260–265. (https://doi.org/10.3109/09513590.2013.871517)

Sabry R, Nguyen M, Younes S & Favetta LA 2022 BPA and its analogs increase oxidative stress levels in in vitro cultured granulosa cells by altering anti-oxidant enzymes expression. *Molecular and Cellular Endocrinology* **545** 111574. (https://doi.org/10.1016/j.mce.2022.111574)

Sadowski RN, Park P, Neese SL, Ferguson DC, Schantz SL, Juraska JM 2014 Effects of perinatal bisphenol A exposure during early development on radial arm maze behavior in adult male and female rats. *Neurotoxicology and Teratology* **42** 17-24. (https://doi.org/10.1016/j.ntt.2014.01.002)

Saleh AC, Sabry R, Mastromonaco GF & Favetta LA 2021 BPA and BPS affect the expression of anti-Mullerian hormone (AMH) and its receptor during bovine oocyte maturation and early embryo development. *Reproductive Biology and Endocrinology : RB&E* **19** 119. (https://doi.org/10.1186/s12958-021-00773-6)

Samardzija D, Pogrmic-Majkic K, Fa S, Stanic B, Jasnic J & Andric N 2018 Bisphenol A decreases progesterone synthesis by disrupting cholesterol homeostasis in rat granulosa cells. *Molecular and Cellular Endocrinology* **461** 55–63. (https://doi.org/10.1016/j.mce.2017.08.013)

Sang C, Song Y, Jin TW, Zhang S, Fu L, Zhao Y, Zou X, Wang Z, Gao H & Liu S 2021 Bisphenol A induces ovarian cancer cell proliferation and metastasis through estrogen receptor-α pathways. *Environmental Science and Pollution Research International* **28** 36060–36068. (https://doi.org/10.1007/s11356-021-13267-0)

Segovia-Mendoza M, Gómez de León CT, García-Becerra R, Ambrosio J, Nava-Castro KE & Morales-Montor J 2020 The chemical environmental pollutants BPA and BPS induce alterations of the proteomic profile of different phenotypes of human breast cancer cells: a proposed interactome. *Environmental Research* **191** 109960. (https://doi.org/10.1016/j.envres.2020.109960)

Sharpe RM & Franks S 2002 Environment, lifestyle and infertility--an inter-generational issue. *Nature Cell Biology* **4** s33–s40. (https://doi.org/10.1038/ncb-nm-fertilityS33)

Shi J, Liu C, Chen M, Yan J, Wang C, Zuo Z & He C 2021 The interference effects of bisphenol A on the synthesis of steroid hormones in human ovarian granulosa cells. *Environmental Toxicology* **36** 665–674. (https://doi.org/10.1002/tox.23070)

Silvestris E, Cohen M, Cornet D, Jacquesson-Fournols L, Clement P, Chouteau J, Schneider M, Besnard T & Ménézo Y 2017 Supporting the One-Carbon Cycle Restores Ovarian Reserve in Subfertile Women: Absence of Correlation with Urinary Bisphenol A Concentration. *BioResearch Open Access* **6** 104–109. (https://doi.org/10.1089/biores.2017.0016)

Singh S & Li SS-L 2012 Epigenetic effects of environmental chemicals bisphenol A and phthalates. *International Journal of Molecular Sciences* **13** 10143–10153. (https://doi.org/10.3390/ijms130810143)

Soccio RE & Breslow JL 2003 StAR-related lipid transfer (START) proteins: mediators of intracellular lipid metabolism. *Journal of Biological Chemistry* **278** 22183–22186. (https://doi.org/10.1074/jbc.R300003200)

Song D, Wu G, Wei Q & Shi F 2019 Bisphenol A attenuates thyroxine-induced apoptosis in ovarian granulosa cells of pigs. *Reproduction in Domestic Animals* **54** 864–872. (https://doi.org/10.1111/rda.13436)

Soundararajan A, Prabu P, Mohan V, Gibert Y & Balasubramanyam M 2019 Novel insights of elevated systemic levels of bisphenol-A (BPA) linked to poor glycemic control, accelerated cellular senescence and insulin resistance in patients with type 2 diabetes. *Molecular and Cellular Biochemistry* **458** 171–183. (https://doi.org/10.1007/s11010-019-03540-9)

Souter I, Smith KW, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM & Hauser R 2013 The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments. *Reproductive Toxicology* **42** 224-231. (https://doi.org/10.1016/j.reprotox.2013.09.008)

Supornsilchai V, Jantarat C, Nosoognoen W, Pornkunwilai S, Wacharasindhu S & Soder O 2016 Increased levels of bisphenol A (BPA) in Thai girls with precocious puberty. *Journal of Pediatric Endocrinology and Metabolism* **29** 1233-1239. (https://doi.org/10.1515/jpem-2015-0326)

Stavridis K, Triantafyllidou O, Pisimisi M & Vlahos N 2022 Bisphenol-A and Female Fertility: An Update of Existing Epidemiological Studies. *Journal of Clinical Medicine* **11** 7227. (https://doi.org/10.3390/jcm11237227)

Tamrakar SR & Bastakoti R 2019 Determinants of infertility in couples. *Journal of Nepal Health Research Council* **17** 85–89. (https://doi.org/10.33314/jnhrc.1827)

Tarafdar A, Sirohi R, Balakumaran PA, Reshmy R, Madhavan A, Sindhu R, Binod P, Kumar Y, Kumar D & Sim SJ 2022 The hazardous threat of Bisphenol A: Toxicity, detection and remediation. *Journal of Hazardous Materials* **423** (Part A) 127097. (https://doi.org/10.1016/j.jhazmat.2021.127097)

Téteau O, Jaubert M, Desmarchais A, Papillier P, Binet A, Maillard V & Elis S 2020 Bisphenol A and S impaired ovine granulosa cell steroidogenesis. *Reproduction* **159** 571–583. (https://doi.org/10.1530/REP-19-0575)

Tsutsumi O 2005 Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction. *Journal of Steroid Biochemistry and Molecular Biology* **93** 325–330. (https://doi.org/10.1016/j.jsbmb.2004.12.008)

Vahedi M, Saeedi A, Poorbaghi SL, Sepehrimanesh M & Fattahi M 2016 Metabolic and endocrine effects of bisphenol A exposure in market seller women with polycystic ovary syndrome. *Environmental Science and Pollution Research International* **23** 23546-23550. (https://doi.org/10.1007/s11356-016-7573-5)

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee D-H, Shioda T, Soto AM, vom Saal FS, Welshons WV et al. 2012 Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews* **33** 378–455. (https://doi.org/10.1210/er.2011-1050)

van Gool JD, Hirche H, Lax H & De Schaepdrijver L 2018 Folic acid and primary prevention of neural tube defects: A review. *Reproductive Toxicology* **80** 73–84. (https://doi.org/10.1016/j.reprotox.2018.05.004)

Vasiljevic T & Harner T 2021 Bisphenol A and its analogues in outdoor and indoor air: properties, sources and global levels. *Science of the Total Environment* **789** 148013. (https://doi.org/10.1016/j.scitotenv.2021.148013)

Vom Saal FS & Vandenberg LN 2021 Update on the Health Effects of Bisphenol A: Overwhelming Evidence of Harm. *Endocrinology* **162** bqaa171. (https://doi.org/10.1210/endocr/bqaa171)

Wade M, Delawder V, Reneau P & Dos Santos JM 2020 The effect of BPA exposure on insulin resistance and type 2 diabetes - the impact of muscle contraction. *Medical Hypotheses* **140** 109675. (https://doi.org/10.1016/j.mehy.2020.109675)

Wallace WHB & Kelsey TW 2010 Human ovarian reserve from conception to the menopause. *PLoS One* **5** e8772. (https://doi.org/10.1371/journal.pone.0008772)

Wan MLY, Co VA & El-Nezami H 2021 Endocrine disrupting chemicals and breast cancer: a systematic review of epidemiological studies. *Critical Reviews in Food Science and Nutrition* **62** 6549–6576. (https://doi.org/10.1080/10408398.2021.1903382)

Wang Y, Zhu Q, Dang X, He Y, Li X & Sun Y 2017 Local effect of bisphenol A on the estradiol synthesis of ovarian granulosa cells from PCOS. *Gynecological Endocrinology* **33** 21–25. (https://doi.org/10.1080/09513590.2016.1184641)

Watanabe M, Ohno S & Nakajin S 2012 Effects of bisphenol A on the expression of cytochrome P450 aromatase (CYP19) in human fetal osteoblastic and granulosa cell-like cell lines. *Toxicology Letters* **210** 95–99. (https://doi.org/10.1016/j.toxlet.2012.01.020)

Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT & Belcher SM 2007 In vitro molecular mechanisms of bisphenol A action. *Reproductive Toxicology* **24** 178–198. (https://doi.org/10.1016/j.reprotox.2007.05.010)

Wehbe Z, Nasser SA, El-Yazbi A, Nasreddine S & Eid AH 2000 Estrogen and Bisphenol A in Hypertension. *Current Hypertension Reports* **22** 23. (https://doi.org/10.1007/s11906-020-1022-z)

Wiersielis KR, Samuels BA & Roepke TA 2020 Perinatal exposure to bisphenol A at the intersection of stress, anxiety, and depression. *Neurotoxicology and Teratology* **79** 106884. (https://doi.org/10.1016/j.ntt.2020.106884)

Wu G, Song D, Wei Q, Xing J, Shi X & Shi F 2018 Melatonin mitigates bisphenol A-induced estradiol production and proliferation by porcine ovarian granulosa cells in vitro. *Animal Reproduction Science* **192** 91–98. (https://doi.org/10.1016/j.anireprosci.2018.02.018)

Wu W, Li M, Liu A, Wu C, Li D, Deng Q, Zhang B, Du J, Gao X & Hong Y 2020 Bisphenol A and the risk of obesity a systematic review with meta-analysis of the epidemiological evidence. *Dose-Response* **18** 1559325820916949. (https://doi.org/10.1177/1559325820916949)

Wu M, Wang S, Weng Q, Chen H, Shen J, Li Z, Wu Y, Zhao Y, Li M, Wu Y *et al.* 2021 Prenatal and postnatal exposure to bisphenol A and Asthma: a systemic review and meta-analysis. *Journal of Thoracic Disease* **13** 1684–1696. (https://doi.org/10.21037/jtd-20-1550)

Xu J, Osuga Y, Yano T, Morita Y, Tang X, Fujiwara T, Takai Y, Matsumi H, Koga K, Taketani Y *et al.* 2002 Bisphenol A induces apoptosis and G2-to-M arrest of ovarian granulosa cells. *Biochemical and Biophysical Research Communications* **292** 456–462. (https://doi.org/10.1006/bbrc.2002.6644)

Xu G, Han A, Xu N & Su P 2018 [Effects of maternal exposure to bisphenol A during pregnancy on puberty in advance and hypothalamo-pituitary gonadal axis hormones level in female offspring]. *Wei Sheng Yan Jiu* **47** 425-431.

Yamada Y & Satoh K 1997 Folliculogenesis and regulating factors. *Nihon Rinsho. Japanese Journal of Clinical Medicine* **55** 2822–2828.

Zhang H, Vollmer M, De Geyter M, Litzistorf Y, Ladewig A, Dürrenberger M, Guggenheim R, Miny P, Holzgreve W & De Geyter C 2000 Characterization of an immortalized human granulosa cell line (COV434). *Molecular Human Reproduction* **6** 146–153. (https://doi.org/10.1093/molehr/6.2.146)

Zhou W, Liu J, Liao L, Han S & Liu J 2008 Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. *Molecular and Cellular Endocrinology* **283** 12–18. (https://doi.org/10.1016/j.mce.2007.10.010)

Zhou W, Fang F, Zhu W, Chen ZJ, Du Y & Zhang J 2016 Bisphenol A and Ovarian Reserve among Infertile Women with Polycystic Ovarian Syndrome. *International Journal of Environmental Research and Public Health* **14** 18. (https://doi.org/10.3390/ijerph14010018)

Ziv-Gal A & Flaws JA 2016 Evidence for bisphenol A-induced female infertility: a review (2007–2016) (2007–2016). *Fertility and Sterility* **106** 827–856. (https://doi.org/10.1016/j.fertnstert.2016.06.027)

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